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Modulation of cardiovascular risk factors and inflammation in rheumatic diseases

Izhar Chaim van Eijk

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promotoren: prof.dr. B.A.C. Dijkmans

 prof.dr. Y.M. Smulders

copromotoren: dr. M.T. Nurmohamed

 dr. D. van Schaardenburg

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GENERAL INTRODUCTION

This thesis focuses on two main subjects:

1. Effects of anti-inflammatory therapy on cardiovascular risk factors in patients with either rheumatoid arthritis (RA) or ankylosing spondylitis (AS).
2. Comparison of two treatment strategies in early inflammatory arthritis and influence of strong anti-inflammatory therapy on cell-derived microparticles in early RA.

The relationship between atherosclerosis and inflammation and additionally the link between cardiovascular disease and rheumatic diseases will be explained in the first part of the introduction. Also, some of the important traditional and new cardiovascular risk factors are introduced in light of the studies presented in this thesis. The second part of the introduction focuses on the evolution on treatment of RA and undifferentiated arthritis and introduces microparticles.

SECTION 1

INFLAMMATION, ATHEROSCLEROSIS AND INFLAMMATORY RHEUMATIC DISEASE

Atherosclerosis and inflammation

Nowadays, atherosclerosis, the leading cause of morbidity and mortality in the Western society, is considered an inflammatory disease (1-3). Since the 1990s more than a dozen large-scale prospective cohort studies indicate that low-grade inflammation (as measured by C-reactive protein) predicts incident myocardial infarction, stroke, and cardiovascular death even after full adjustment for the traditional cardiovascular risk factors, such as smoking, hypertension, cholesterol and diabetes (4-7). Based on this knowledge, many similarities were demonstrated between inflammation mechanisms and atherosclerotic lesions: immune cells appear to dominate early atherosclerotic lesions, their effector molecules accelerate progression of the lesions, and inflammation can elicit acute coronary syndromes (8). It has become increasingly clear that inflammation plays a pivotal role in atherogenesis in all stages of its development (8;9), from endothelial dysfunction, fatty streak formation and deterioration to eventually plaque rupture (10-12). In addition, systemic inflammation originating primarily outside the vascular system can accelerate atherosclerosis through the amplification of effects of conventional and novel cardiovascular risk factors, such as hyperlipidemia, hypertension, and insulin resistance (13-15). Against this background, individuals with clinically apparent chronic inflammatory conditions, such as rheumatoid arthritis (RA) and

ankylosing spondylitis (AS), are of particular interest to investigate the relationship between inflammation and atherosclerosis.

Inflammatory rheumatic diseases and cardiovascular disease

Two of the most common inflammatory rheumatic diseases are RA and AS. RA is a chronic systemic inflammatory disease of unknown aetiology affecting approximately 1% of the general population (16;17). The main clinical feature of RA is a symmetric polyarthritis of in particular the small joints of hands and feet that can result in destruction of cartilage and bone. In RA, standardized mortality ratios are elevated and life expectancy is shortened by 3-18 years (18;19). Cardiovascular disease drives much of the excess morbidity and mortality risk in patients with RA (20;21) and the relative risk of (fatal and non-fatal) cardiovascular disease is approximately 2-4 times higher in RA compared to the general population (22-26).

AS also is a chronic inflammatory rheumatic disease. It has a prevalence around 0.5% of the general population, affects predominantly males, starting in young adulthood and potentially results in immobility of the sacroiliac joints and spine (27). Patients with AS have an approximately twofold increased mortality rate as compared to the general population, which is predominately caused by cardiovascular diseases (28-30). Although specific cardiovascular disorders (valvular disease and conduction disturbances) occur more frequently in AS (30;31), accelerated atherosclerotic disease probably contributes to the increased cardiovascular risk as well (32;33). Recently it was demonstrated that in AS the prevalence rate for myocardial infarction is approximately twofold to threefold increased in comparison with the general population (34). Impaired endothelial function, as a surrogate marker for atherosclerosis development, has been demonstrated in peripheral arteries as well as in the coronary microcirculation of AS patients, and the severity of these impairments correlated well with inflammatory parameters (35;36). These observations indicate that AS, like RA, should be considered as an important CV risk factor.

Cardiovascular risk factors

The increased cardiovascular risk in both RA and AS may be due to a higher prevalence of traditional cardiovascular risk factors. Moreover, as will be discussed below, evidence is accumulating that inflammation is an independent cardiovascular risk factor, and additionally amplifies the pro-atherogenic effects of other (traditional) cardiovascular risk factors. Some important cardiovascular risk factors in relation to inflammation are introduced below.

Dyslipidemia. An established risk factor for atherosclerosis is an atherogenic lipid profile. Particularly low levels of high-density lipoprotein cholesterol (HDLc), and high levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDLc), and triglycerides (TG) are associated with an increased cardiovascular risk in the general population (37;38). The “atherogenic index”, which is the ratio of TC to HDL, is often used in studies as it was shown to be an important prognostic indicator for cardiovascular disease (39). More recently, another indicator based on apolipoproteins, which are protein subunits of the lipoprotein complex, has emerged. High levels of apolipoprotein B (almost exclusively expressed as a component of LDL cholesterol-containing lipoproteins) and an increased apolipoprotein B / apolipoprotein A-1 ratio are associated with an increased cardiovascular risk, whereas higher levels of apolipoprotein A-1 (primarily expressed on HDLc) are protective for developing cardiovascular disease (40-42). Indeed, several investigations have reported that patients with RA or AS, particularly those with high inflammatory activity, have a deteriorated lipid profile, characterized by lower HDLc, TC, and apoA-1 levels, increased levels of LDLc, TG and apoB and a higher (i.e. unfavourable) HDLc/TC ratio compared to healthy control subjects (43-48). In addition to changes in lipid levels, the actual composition of lipoproteins may be important. New data indicate that inflammation can affect HDL qualitatively (49). During inflammation specific enzyme and protein components of HDL, contributing to HDL’s (anti)atherogenic potential, such as serum amyloid A protein (SAA) and apoA-1, are modified and may even render the particle proatherogenic (50). Therefore, in the context of inflammation, both absolute levels of lipids as well as the composition of specific lipoproteins seem important.

Hypertension. At present, hypertension is defined as a systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg. Regardless of its definition, a continuous positive correlation between arterial pressure and cardiovascular risk has been described (51). Blood pressure is influenced by many factors. Essential hypertension is characterized by a normal cardiac output and an elevation in peripheral vascular resistance (52). Peripheral vascular resistance is primarily dictated by resistance across the smallest vessels i.e. arterioles, capillaries and venules (53;54), which is known as the microcirculation. The microcirculation has several functions, such as to supply oxygen and nutrients, to exchange waste products with tissues, and to avoid inappropriate fluctuations in hydrostatic capillary pressure (55). Hypertension is characterized by both structural and functional microvascular defects, such as enhanced vasoconstriction and reduced vasodilator responses to provocative stimuli and a reduction in the density, so-called rarefaction, of arterioles and capillaries (56-59). It has been shown that microvascular alterations can be secondary to a sustained high blood pressure (53), but it is also suggested that abnormalities in the

microcirculation may precede and thus may be a causal component of an elevated blood pressure. Recent observations suggest that inflammation can also influence microvascular function, as described later (36;60;61). Another factor affecting blood pressure is arterial compliance. Arterial stiffness is associated with inflammation, which as a consequence may cause an increased blood pressure, particularly in inflammatory arthritis patients (13;62).

Diabetes. Heart disease, particularly coronary heart disease is a major cause of morbidity and mortality among patients with diabetes mellitus (63). The National Cholesterol Education Program Guidelines from the United States and guidelines from Europe consider type 2 diabetes to be a coronary heart disease equivalent, thereby elevating it to the highest risk category (64;65). Insulin resistance is an important pathophysiological mechanism in the development of cardiovascular disease (66). The proinflammatory cytokine tumour necrosis factors α (TNF α) has been closely linked to obesity and insulin resistance (67). Adipose tissue is one of the places where TNF α is synthesized and secreted, and increased TNF α plasma levels are associated with long-term glycaemic control (68). TNF α has several harmful effects, as it reduces insulin signaling, causes insulin resistance through decreasing the tyrosine kinase activity of the insulin receptor, and disturbs the insulin-mediated glucose disposal in the skeletal muscle (69-71). Increased production of TNF α is associated with obesity-related insulin resistance (70;72) and administration of TNF α to animals induces insulin resistance (73-75), whereas neutralization of TNF α improved insulin sensitivity (70;76). TNF α levels are also markedly increased in inflammatory diseases, such as RA and AS, which suggests that inflammation may cause insulin resistance or diabetes via this mechanism (77).

Metabolic syndrome. "Metabolic syndrome" is the co-occurrence of metabolic risk factors for both type 2 diabetes and cardiovascular disease (abdominal obesity, hyperglycemia, dyslipidemia (low HDL cholesterol and high triglycerides (TG) levels), and hypertension) (78-81). Genetic predisposition, lack of exercise, and body fat distribution all affect the likelihood that a given obese subject will become overtly diabetic or develop cardiovascular disease.

In this thesis the metabolic syndrome was defined according to the original National Cholesterol Education Program – Third Adult Treatment Panel (NCEP ATP III) definition (82). According to this definition patients fulfil the criteria for metabolic syndrome when three or more of the following factors are present:

- o Abdominal obesity: in females waist circumference > 88 cm, and in males waist circumference > 102 cm;
- o Raised blood pressure: systolic blood pressure \geq 130 mmHg or diastolic blood pressure \geq 85 mmHg;

- o Raised TG: ≥ 1.7 mmol/l (150 mg/dl);
- o Reduced HDL cholesterol: in females < 1.29 mmol/l (50 mg/dl), and in males < 1.03 mmol/l (40 mg/dl);
- o Raised fasting plasma glucose: ≥ 6.1 mmol/l (110 mg/dl).

Patients with metabolic syndrome are at increased risk for cardiovascular events (83). However, it is important to realize that, although metabolic syndrome predicts future cardiovascular disease risk, literature suggests that metabolic syndrome does not enhance the predictive power for cardiovascular disease of established scoring algorithms as the Framingham score. Moreover, questions have been raised as to whether the metabolic syndrome, as currently defined, captures any unique pathophysiology implied by calling it a "syndrome" (84). The advantage of metabolic syndrome is that it serves well as a simple clinical tool for identifying high-risk subjects predisposed to cardiovascular disease (85;86). Regardless of whether the metabolic syndrome is considered a unique entity, the need to identify and manage its individual components is unquestioned.

Inflammation. As discussed earlier, over the past few years our understanding of the importance of inflammation during all stages of atherosclerosis, from its inception through its progression and its final complication of thrombosis, has greatly increased. Atherosclerosis is now considered a disease with an important inflammatory component. Many parallels exist between atherosclerosis and inflammatory processes (8;87-89). Under normal conditions, the endothelial cells of the arterial wall resist adhesion and aggregation of leukocytes and promote fibrinolysis. When activated by stimuli such as proinflammatory cytokines (IL-1, IL-6, and TNF α) that are highly present in RA and AS, the endothelial cells express a series of adhesion molecules (e.g. ICAM and VCAM) that selectively recruit various classes of leukocytes and T-lymphocytes. Monocytes adhere to the activated endothelium and enter the intima. Within the intima, the monocytes mature into macrophages, engulf modified lipoprotein particles and develop into foam cells (1;87;88;90-95). The progression of fatty streaks to more complex lesions normally occurs over many years and the rate of progression is dependent on the presence of several inflammatory mediators in the arterial wall. Systemic inflammation as present in RA and AS may accelerate the atherosclerotic process by providing cytokines to the circulation and thereby stimulating the processes described above. It was demonstrated that inflammation regulates both the "solid-state" thrombotic potential in the plaque itself and the prothrombotic and antifibrinolytic capacity of blood in the fluid phase (96). Histological evidence has revealed that lesions from RA patients not only yield more inflammatory components but also more features of instability as

compared to controls (97). In addition to these direct effects of inflammation on development of atherosclerosis, inflammation probably also increases cardiovascular risk through deteriorating known cardiovascular risk factors (98). Therefore, it can be expected that patients having inflammatory disease are more prone to develop cardiovascular disease.

Endothelial Dysfunction. Endothelial dysfunction precedes and initiates atherosclerosis and is a predictor of long-term cardiovascular risk (99). Endothelial dysfunction is commonly detected by impairment of endothelial-dependent vasodilatation. In intact endothelium the production of nitric oxide (NO), a potent vasodilator, causes relaxation of the vascular smooth muscle. NO, produced by endothelial nitric oxide synthase (eNOS), is a fundamental determinant of cardiovascular homeostasis: it regulates blood pressure, vascular remodeling and angiogenesis. Increased TNF α levels may have an important role in the pathogenesis of endothelial dysfunction in inflammatory diseases, such as RA and AS (36;100;101). TNF α blocks the activation of eNOS, which is essential for flow-dependent vasodilatation, by directly degrading eNOS mRNA and through inhibition of insulin mediated action of insulin receptor substrate-1, PI3-kinase and AKT (102-105). In-vitro studies suggest that TNF blockade reduces endothelial activation and down regulates expression of E-selectin and vascular cell adhesion molecule-1 (VCAM-1) (106). This may be one of the additional mechanisms through which inflammation directly accelerates atherosclerosis.

Vascular dysfunction has also been studied to explain the occurrence of traditional cardiovascular risk factors. As discussed earlier, a particularly interesting type of vascular dysfunction occurs in the microvasculature. Recent advances have highlighted the crucial involvement of the microcirculation in many cardiovascular conditions, such as the development of target-organ damage in the heart and kidney. In addition, microvascular dysfunction is closely, and presumably causally, linked to particularly insulin resistance and hypertension (107;108). Summarizing, vascular dysfunction may well be an additional pathway resulting in accelerated atherosclerosis as well as a mechanical explanation for the development of traditional cardiovascular risk factors, particularly hypertension and insulin resistance.

SECTION 2

EARLY INFLAMMATORY ARTHRITIS

In the late 1990s inception cohorts were set up in various countries to study the features and outcome of early inflammatory polyarthritis. The aim of these “Early Arthritis Clinics” was to minimize delay in referral, in order to start adequate treatment in early to minimize joint destruction and disability. This development was a consequence of reported favourable

outcomes of early aggressive treatment of RA, particularly with combinations of disease modifying antirheumatic drugs (DMARD), also containing anti-TNF therapy (109-113). The most impressive results are high percentages of sustained (drug-free) remission, excellent functional status and nearly complete arrest of radiological damage progression. In an attempt to explain these favourable outcomes of early treatment, the concept of a “window of opportunity” was proposed, suggesting that early suppression of active inflammation produces long-term benefits (114).

In patients with active polyarthritis, aggressive treatment is justified by the high joint count, and the presence of other prognostic factors is not decisive (115). Also, depending on the severity of the disease, a certain degree of treatment associated toxicity is acceptable. In patients presenting with only a few inflamed joints, however, the optimal treatment strategy is currently not clear. This category of patients is more difficult to study, partly because it is more difficult to demonstrate a reduction of already low disease activity, and partly because in patients presenting with milder disease any toxicity of treatment is less acceptable. In addition, there is often a problem of classifying these patients as having either RA or undifferentiated arthritis (UA) (116). Prognosis in patients with UA varies. In the first one to two years, most UA patients will either progress to RA (10-40%) or remit (around 50%) (117-120).

In clinical practice, the diagnosis RA is still largely based on the expert opinion of the rheumatologist. In recent years, the presence of anti-citrullinated protein antibodies (ACPA) has emerged as a major new biomarker for use in clinical practice. It can be used to divide patients with early arthritis into subsets that are phenotypically similar but have varying pathogenetic and prognostic features (121;122). The presence of ACPA has the highest sensitivity and specificity for development of RA and is associated with worse outcome (122-124). Other predictors for a poor outcome are the presence of rheumatoid factor (RF) and of the shared epitope at the HLA-DRB1 locus (125;126). Nowadays, diagnosing RA according to the 1987 American College of Rheumatology (ACR) criteria for RA (127) is not very helpful, since these criteria have been shown to be relative insensitive in patients with a symptom duration of less than one year (128;129). At first presentation nodules and radiographic damage often are not present and the criteria of synovitis may be shared by self-limiting forms of polyarthritis (130;131). Moreover, when synovitis is suppressed by early treatment, as now often occurs: according to the ACR criteria one can then no longer speak of RA. Therefore, very recently, new ACR/EULAR criteria for classification of RA were launched, presenting a new approach with a specific emphasis on identifying patients with a relatively short duration of symptoms who may benefit from early institution of DMARD therapy. The aim is to provide a standardized approach for discriminating, from a population

of individuals presenting with undifferentiated synovitis, the subgroup with the highest probability of persistent or erosive RA (132). Logically, as a consequence of the data described above, ACPA status is implemented in these new criteria.

Summarizing, at present, there are a number of important challenges for clinicians treating early arthritis. The first is how to make an accurate selection of patients who have a poor prognosis and need intensive treatment versus those who have a good prognosis and need mainly symptomatic treatment. The second challenge is to achieve remission in most patients, preferably drug-free. The third is to establish whether the aggressive approach in active RA is also effective in a milder disease category, since in this subgroup of patients toxicity is less acceptable.

Cell derived microparticles

Recently, cell-derived microparticles (MP), which are small membrane vesicles released from blood cells or endothelial cells upon activation or during apoptosis, have emerged as new pro-inflammatory mediators. MP are thought to amplify or disseminate inflammation. MP were shown to be associated with complement activation, inflammation and coagulation in various diseases, including inflammatory diseases (133-137). They are thought to trigger inflammation by several processes such as activation of endothelial cells and leukocytes, causing production and release of chemokines and cytokines, and by activating the complement cascade which is thought to play a key role in the pathogenesis of RA (134;138-143).

Conversely, inflammation may trigger MP formation. For instance, in vitro studies showed that MP are released from cells incubated with TNF α or IL-1, and a study in mice showed that the number of platelet-derived MP in plasma markedly increased upon injection with TNF α (144-146). Although inflammation causes release of MP and in turn MP may induce or enhance inflammation, it remains unknown whether circulating MP merely reflect ongoing inflammation or whether MP actually contribute to the disease development.

MP are also linked to cardiovascular disease. Elevated levels of platelet-derived MP circulate in patients at risk for thromboembolic complications (147). In addition, it was demonstrated platelet-derived MP subpopulations (CD62p or CD63), which reflect platelet activation better than the total numbers of circulating platelet-derived MP, are increased in patients with peripheral artery disease or myocardial infarction (148). Finally it is suggested that MP interfere with vascular function (149-151).

THESIS OUTLINE

The purpose of this thesis is to gain more insight in the underlying mechanisms of the elevated cardiovascular risk in patients with inflammatory rheumatic diseases, focussing on traditional and new cardiovascular risk factors. In addition, the effects of powerful anti-inflammatory treatment (anti-TNF therapy) on these risk factors are being studied.

In section 3, the efficacy of two different treatment strategies in early inflammatory arthritis is evaluated. Also, to determine whether circulating MP numbers are associated with inflammatory activity in RA patients, MP in very early yet untreated arthritis patients and healthy controls were compared and additionally, the effects of changes in disease activity upon intense anti-inflammatory therapy on MP numbers and composition were investigated.

SECTION 1 (Chapters 1.1-1.4) cardiovascular risk and role of inflammation in patients with ankylosing spondylitis:

In **chapter 1.1 and 1.2**, the cardiovascular risk in AS patients is assessed through risk factors/surrogate markers, i.e. endothelial function, carotid intima media thickness and arterial stiffness. In addition, the effect of powerful inflammatory suppression (anti-TNF treatment) on microvascular function and on arterial wall properties is examined. **Chapter 1.3** focuses on dyslipidemia and its relationship with inflammation. In particular, the effect of anti-TNF treatment on the composition of HDL particles is studied. In **chapter 1.4**, the efficacy of traditional and new inflammatory markers ESR, CRP, hsCRP and SAA for monitoring inflammation in AS patients treated with anti-TNF along with the association between these inflammatory markers and the BASDAI over time is explored.

SECTION 2 (Chapters 2.1-2.4) cardiovascular risk in patients with rheumatoid arthritis:

Chapter 2.1 examines the prevalence of the metabolic syndrome (and its features) in RA patients with hypothyroidism relative to euthyroid RA patients as a possible explanation of the increased cardiovascular risk observed in hypothyroid RA patients as compared to patients with RA alone. In addition, the Framingham risk score, as an established tool for identifying high-risk individuals, is used to compare the estimated 10-year cardiovascular disease risk in these groups. **Chapter 2.2** describes microvascular function in early DMARD-naïve RA-patients compared to matched controls to establish whether microvascular function is already disturbed in early RA with low systemic inflammation. **Chapter 2.3** reports on two

cases of RA patients with concomitant diabetes, in which glycaemic control parameters beneficially changed after initiation of TNF blocking therapy.

SECTION 3 (Chapter 3.1-3.2) Treatment strategy in patients with early inflammatory arthritis: **Chapter 3.1** investigates whether early aggressive treatment, which has been shown useful in active RA, is also effective in arthritis patients presenting with only moderately active disease, i.e. in those patients who would not meet the usual inclusion criteria for trials in active RA. **Chapter 3.2** determines the association of circulating MP with inflammatory activity and the effect of strong anti-inflammatory therapy on MP numbers and composition.

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SECTION 1

Cardiovascular risk in patients with ankylosing spondylitis

Chapter 1.1

Signs of accelerated preclinical atherosclerosis in patients with ankylosing spondylitis

M.J.L. Peters
I.C. van Eijk
Y.M. Smulders
E.H. Serné
B.A.C. Dijkmans
I.E. van der Horst-Bruinsma
M.T. Nurmohamed

ABSTRACT

Objective. Preliminary evidence suggests that ankylosing spondylitis (AS) is associated with an increased cardiovascular (CV) risk. We investigated subclinical atherosclerosis and arterial stiffness in patients with AS compared with control subjects, and identified CV and AS related risk factors for atherosclerotic disease.

Methods. 59 AS patients, who were scheduled for etanercept treatment according to the ASAS guidelines, and 30 healthy controls were recruited. Subclinical atherosclerosis was assessed as the average intima-media thickness (IMT) of the common carotid artery. Arterial stiffness was determined by distensibility, compliance and Young's elastic modulus of the carotid artery.

Results. AS patients had a greater IMT (0.62 ± 0.09 mm versus 0.57 ± 0.09 mm, $p=0.02$), that remained after adjustment for traditional CV risk factors. In addition, AS was associated with a higher carotid pulse pressure (47 ± 7 mmHg versus 44 ± 8 mmHg, $p=0.04$), but this was not due to local vessel wall properties. Among AS patients, age and body mass index were determinants of IMT. Age, body mass index, total cholesterol, triglycerides and disease duration were identified as determinants of stiffness indices. No relationship was found between large vessel properties on the one hand, and higher bath ankylosing spondylitis indices or C-reactive protein values on the other.

Conclusions. This study demonstrates that AS is associated with subclinical atherosclerosis and arterial stiffness supporting epidemiological evidence of an increased CV risk in these patients. Whether these differences are truly due to AS or due to a higher prevalence of CV risk factors in AS patients needs to be addressed in future studies.

INTRODUCTION

Ankylosing spondylitis (AS), a chronic inflammatory disease of the sacroiliac joints and spine affecting up to 1% of the population, is associated with increased mortality rates (1). Epidemiological studies have shown that this increased mortality is largely attributable to cardiovascular (CV) disease (2-4). We recently found that, in a large sample of AS patients aged between 50 and 75 years, the prevalence of myocardial infarction is increased by approximately 2-3 fold compared with control subjects matched for age and gender (5). Accelerated atherosclerosis in AS may be due to traditional CV risk factors, e.g. an atherogenic lipid profile and hypertension (6;7). In addition, the generalized inflammatory state that characterizes active AS may render these patients more prone to develop CV disease (8). In fact, there is increasing evidence that the underlying inflammatory process in chronic inflammatory conditions resembles the chronic inflammatory processes that contribute to various stages of atherothrombosis, from early atheroma formation to plaque instability and thrombus formation (9;10).

High-resolution ultrasonography can be used to measure the intima-media thickness (IMT) as well as vascular elasticity of the carotid artery. An increased carotid IMT reflects the atherosclerotic burden and predicts the development of (clinically apparent) CV disease in the general population (11;12). Arterial stiffness is another important and independent risk factor for CV disease, partly due to its association with increased systolic blood pressure, ventricular mass, and decreased diastolic coronary perfusion (13-16).

It is currently unknown whether AS patients without CV disease show early signs of large artery damage compared to controls and, if so, what the determinants of such large vessel abnormalities are. This knowledge could prove useful for future development of risk stratification and intervention strategies. Hence, this study was designed to determine whether signs of subclinical atherosclerosis and arterial stiffness are more prominent in a sample of AS patients compared to healthy controls, and to study determinants of these large vessel characteristics.

PATIENTS AND METHODS

Study population

Eighty-two consecutive AS patients attending the outpatient clinic of the Jan van Breemen Institute and VU University Medical Center and who were scheduled to receive etanercept treatment according to the ASAS guidelines for anti-TNF α treatment were studied (17). All patients fulfilled the modified New York diagnostic criteria of AS (18). Thirty volunteers

(patients' buddies or hospital staff) matched for age and gender served as controls. Patients and controls were excluded if a CV disease history was positive (myocardial infarction, percutaneous transluminal coronary angioplasty, surgery for ischemic heart disease, stroke, transient ischemic attack, carotid endarterectomy, peripheral arterial reconstructive surgery or limb amputation). In addition, patients and controls with diabetes mellitus (self-reported or when using glucose lowering agents), hypertension (systolic blood pressure over 140 mmHg, diastolic blood pressure over 90 mmHg, or antihypertensive usage), statin usage, anticoagulants and/or low dose aspirin usage were excluded. At conclusion of the protocol 59 of the 82 AS patients fulfilled the criteria. All participants gave written informed consent and the Institutional Ethics Committee of both hospitals approved the study protocol.

Other measurements

AS patients and controls attended the outpatient clinic at the Jan van Breemen Institute or VU University Medical Center, where they were examined by a research physician, and completed a questionnaire recording demographic data, medical, and medication history. CV risk factors (current smoking (yes/no), blood pressure, body mass index, (self-reported) diabetes), and blood samples (C-reactive protein (CRP), lipids) were assessed in AS patients as well as in control subjects. Height and weight were measured barefoot wearing light clothes only. The mean value of 2 measurements was used. The body mass index was calculated as the ratio of weight and squared height. Serum total cholesterol, HDL (high-density lipoprotein) cholesterol and triglyceride levels were analyzed by enzymatic techniques as described previously (19). The atherogenic index was calculated dividing total cholesterol levels by HDL cholesterol levels. AS related variables that were assessed included: the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) (20), the Bath Ankylosing Spondylitis Functional Index (BASFI) (21), and the AS mobility score (the Bath Ankylosing Spondylitis Metrology Index (BASMI)) (22).

Arterial properties

Measurements were conducted in a quiet, temperature-controlled room after 15 minutes of rest, with the subjects in supine position. Subjects were asked to refrain from beverages other than water (in particular no caffeine or alcohol), smoking, and meals from midnight at the testing day. Brachial systolic, diastolic blood pressures and heart rate were assessed in the left upper arm at 5-minute intervals with an oscillometric device (Collin Press-Mate, BP-8800, Komaki-City, Japan). Brachial pulse pressure (PP) was calculated as systolic minus diastolic blood pressure, and brachial mean arterial pressure (MAP) as $(2 \text{ diastolic pressure} + \text{systolic pressure})/3$.

Ultrasound analysis of the right common carotid artery and the right brachial artery was performed by two observers who were unaware of the participants' clinical or laboratory characteristics. Measurements were performed with a 7.5-MHz linear probe, connected to a PC equipped with vessel wall movement detection software and an acquisition system (Wall track system, Pie Medical, Maastricht, The Netherlands), which enables measurement of the IMT, diameter and distension. The distance between the lumen-intima interface and the leading edge of the media-adventitia interface of the far wall corresponds with IMT. After localization of the common carotid artery, cross-sectional measurements were performed 10 mm proximal of the carotid bulb. Sites with mural atherosclerotic plaque were excluded while measuring. We defined plaques as focal widening of the vessel wall of 50% relative to adjacent segments with protrusion into the lumen. The protrusion was evaluated by eyeballing judgment (23). The mean IMT, diameter, and distension of three consecutive measurements were used for the analyses. Carotid PP was determined according to the method from Van Bortel and coworkers (24). Carotid diameter (D), distension (ΔD), carotid PP (ΔP) and IMT were used to calculate different arterial stiffness indices, as follows (25):

$$\text{- Distensibility coefficient} = (2\Delta D * D + \Delta D^2) / (\Delta P * D^2) \text{ in } 10^{-3} \text{ kPa}^{-1}$$

$$\text{- Compliance coefficient} = \pi(2D * \Delta D + \Delta D^2) / (4 * \Delta P) \text{ in } \text{mm}^2 * \text{kPa}^{-1}$$

$$\text{- Young's Elastic Modulus} = D / (\text{IMT} * \text{distensibility}) \text{ in kPa}$$

Distensibility as defined above reflects the relative change in arterial cross-sectional area per unit change in local PP, whereas compliance reflects the absolute change in cross-sectional area per unit change in local PP. Subsequently, the Young's elastic modulus can be calculated with IMT, diameter and distensibility coefficient of the carotid artery, estimating the intrinsic elastic properties of the elastic wall.

The inter-observer variability [$CV = (\text{standard deviation of the mean difference} / \sqrt{2}) / \text{pooled mean}$] for the studied measurements were: IMT, 7%; carotid diameter, 2%; and carotid distension, 7%. The corresponding values for the intra-observer variability were, for observer 1 and 2 respectively: IMT, 6% and 7%; carotid diameter, 2% and 2%; and carotid distension, 6% and 9%.

Statistical Analyses

Data are expressed as mean (SD) or median (interquartile range) as appropriate. Differences in demographic variables, CV risk factors and arterial wall properties between AS patients and control subjects were investigated using Students' T-tests, Mann-Whitney's U-tests and Pearson's Chi Square tests, when appropriate. Univariate and multivariate linear regression models were conducted to investigate differences in arterial wall properties between AS

patients and controls. Multivariate models were conducted according to a forward selection procedure, introducing variables that showed a significant association with the outcome measure (p -value < 0.10). Variables that were entered in the multivariate models were: age, gender, pulse pressure, heart rate, CRP, smoking, and the atherogenic index. Pulse pressure was replaced by MAP if the outcome measure was one of the arterial stiffness indices. As carotid stiffness indices (i.e. carotid distensibility, compliance and elastic modulus) were not normally distributed, data were analysed with the logarithms (natural) of these values and the regression coefficients and confidence intervals were presented using these values. Among AS patients, we investigated the relationship between arterial wall properties, CV and AS risk factors with Pearson correlation or Spearman's rho tests when appropriate. A two-tailed probability value of $P < 0.05$ was considered statistically significant.

RESULTS

AS patients

Table 1 summarizes the baseline characteristics of all AS patients. Median age of AS patients was 39 years and 63% were male. Most AS patients were HLA-B27 positive (88%), median AS disease duration was 9 years, disease activity according to BASDAI score was 6.2, which represents a high disease activity. All AS patients were currently treated with NSAIDs.

AS patients versus controls

CVD risk profile: AS patients smoked more often had a slightly higher systolic blood pressure and a lower diastolic blood pressure, resulting in a significantly higher pulse pressure (Table 1). CRP was significantly higher in AS patients. No significant differences were observed for total cholesterol, HDL cholesterol, atherogenic index, triglycerides, and body mass index.

Carotid Arterial wall properties: Table 1 shows all carotid arterial wall properties in AS patients as compared with controls. AS patients had an increased IMT (0.62 mm in AS patients versus 0.57 mm in controls, $p = 0.02$) (Figure 1). This difference remained when linear regression analyses were done adjusting for potential confounders (Table 2). In conjunction with a higher brachial pulse pressure, the carotid pulse pressure was significantly higher in AS patients (47 ± 7 mmHg versus 44 ± 8 mmHg, $p = 0.04$, respectively). This difference decreased after adjustment for traditional CV risk factors, but remained statistically significant (Table 2). No significant differences were observed for local wall properties reflecting arterial stiffness, inclusive of carotid distensibility, compliance and elastic modulus.

Table 1. Baseline characteristics of AS patients versus controls

	AS patients n = 59	Controls N = 30	p-value
Demographic			
Age (years)	39 (32-44)	42 (29-57)	0.13
Men, %	63	63	0.75
AS related factors			
Disease duration (years)	9 (3-13)	-	
BASDAI (range 0-10)	6.2 (1.3)	-	
BASFI (range 0-10)	5.2 (1.9)	-	
BASMI (range 0-10)	3 (1-4)	-	
HLA-B27, %	88	-	
CV risk factors			
Smoking, %	47	14	< 0.01
Brachial systolic blood pressure (mmHg)	119 (10)	118 (11)	0.55
Brachial diastolic blood pressure (mmHg)	69 (8)	72 (8)	0.07
Brachial mean arterial pressure (mmHg)	86 (7)	86 (8)	0.65
Brachial pulse pressure (mmHg)	51 (6)	46 (7)	0.01
Heart rate (bpm)	65 (10)	59 (10)	0.02
Total cholesterol (mmol/l)	5.0 (1.0)	4.6 (0.8)	0.42
HDL cholesterol (mmol/l)	1.3 (0.3)	1.4 (0.4)	0.21
Atherogenic index	3.9 (1.1)	3.4 (1.1)	0.08
Triglycerides (mmol/l)	1.1 (0.8-1.8)	1.0 (0.8-1.4)	0.62
Body mass index (kg * m ⁻²)	25 (5)	24 (4)	0.23
Weight (kg)	76 (15)	75 (14)	0.55
Height (cm)	176 (11)	176 (9)	0.65
C-reactive protein (mg/l)	8 (3-25)	0.8 (0.5-1.7)	< 0.01
Carotid artery properties			
IMT (mm)	0.62 (0.09)	0.57 (0.10)	0.02
Carotid diameter (mm)	6.95 (0.68)	6.92 (0.73)	0.86
Carotid distension (µm)	561 (180)	500 (162)	0.14
Carotid pulse pressure (mmHg)	47 (7)	44 (8)	0.04
Distensibility coefficient (10 ⁻³ kPa ⁻¹)	26.7 (20.5-31.9)	26.2 (18.6-35.3)	0.91
Compliance coefficient (mm ² * kPa ⁻¹)	1.00 (0.80-1.20)	0.95 (0.67-1.16)	0.80
Young's elastic modulus (kPa)	0.42 (0.35-0.55)	0.46 (0.35-0.60)	0.39

Continuous variables are presented as means with standard deviations (SD) in case of a normal distribution or as medians with interquartile ranges (IQR) in case of a non-normal distribution. Dichotomous variables are presented as number of cases and percentage of total. Variables significantly different are marked with an asterisk.

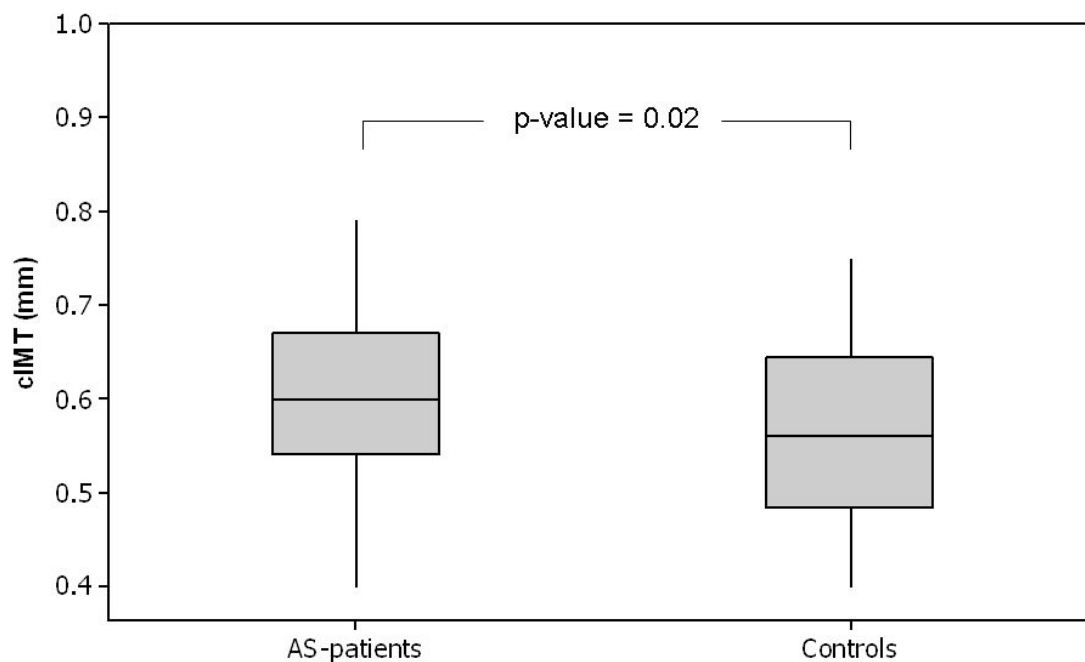


Figure 1. Difference in IMT between AS patients and controls. IMT values from AS patients and controls shown in boxplots: 0.62 mm in AS patients versus 0.57 mm in controls, $p = 0.02$.

Relationship between IMT, arterial stiffness, cardiovascular and AS related risk factors

In AS patients, IMT was correlated with age ($r = 0.392$, $p = 0.002$) and body mass index ($r = 0.288$, $p = 0.03$). IMT did not correlate with AS related risk factors reflecting current disease activity, i.e. BASDAI and CRP, or with AS related risk factors reflecting an increased disease burden, i.e. BASFI, BASMI and disease duration. A subgroup analysis comparing AS patients with CRP levels above 10 mg/l, to those with normal CRP levels also revealed no differences in IMT or arterial stiffness indices (data not shown). In addition, no correlations were observed for carotid pulse pressure with either traditional or AS related risk factors. Arterial stiffness by means of carotid distensibility correlated with age ($r = -0.666$, $p < 0.001$), total cholesterol ($r = -0.522$, $p < 0.001$), triglycerides ($r = -0.416$, $p = 0.001$), and body mass index ($r = -0.284$, $p = 0.03$). In agreement with IMT, arterial stiffness did not correlate with AS related risk factors (CRP, BASDAI, BASFI, and BASMI) except disease duration ($r = -0.362$, $p = 0.005$). Similar correlations were found for the compliance and the elastic modulus (data not shown).

Table 2. Arterial wall properties in AS relative to controls

	B	95%-CI	p-value
IMT			
1: Crude	0.047	0.004 to 0.090	0.021
2: Multivariate (age, gender + CV risk factors)	0.065	0.032 to 0.125	0.001
Carotid pulse pressure			
1: Crude	4.077	0.604 to 7.550	0.040
2: Multivariate	3.588	0.156 to 7.020	0.041
Distensibility coefficient			
1: Crude	0.006	-0.071 to 0.084	0.868
2: Multivariate	-0.021	-0.069 to 0.027	0.388
Compliance coefficient			
1: Crude	0.014	-0.059 to 0.088	0.700
2: Multivariate	0.028	-0.033 to 0.090	0.362
Young's elastic modulus			
1: Crude	-0.043	-0.121 to 0.034	0.271
2: Multivariate	-0.009	-0.065 to 0.048	0.755

Results are expressed as β regression coefficient and 95% confidence intervals (95%CI). As the carotid stiffness indices (carotid distensibility, compliance and elastic modulus) were not normally distributed, data were analysed and presented with the logarithms (natural) of these values. Variables included in the multivariate models were: age, gender, pulse pressure or mean arterial pressure, heart rate, smoking, atherogenic index, and C-reactive protein.

DISCUSSION

There is considerable interest in the association between chronic inflammatory diseases and CV disease, but the current evidence base for a link between AS and CV disease is limited. The present study reveals a higher CV risk among AS patients as indicated by a greater IMT, brachial and carotid pulse pressure in comparison with controls, but this was not reflected by impaired arterial stiffness indices. These observations support epidemiological findings indicating that AS may be associated with an increased CV risk, but obviously further prospective studies are needed to appraise the future clinical relevance of a 0.05mm cIMT difference (5;26). In addition, we observed a high CV risk factor profile in AS patients, and some of these risk factors (lipids and body mass index) were associated with a greater carotid IMT and increased arterial stiffness.

Four studies assessing IMT in AS patients and controls have been published so far (27-30). All studies found a non-significant trend towards a greater IMT in AS patients, but none of these selected AS patients with an active disease as part of the study protocol. Given that inflammation has increasingly been acknowledged as to why rheumatic patients are at elevated CV risk (31), we selected patients with an active disease represented by a BASDAI greater than four at inclusion and insufficient response to NSAIDs (32-34). However, at least

in our cohort, we did not find a relationship between large vessel properties on the one hand, and higher bath ankylosing spondylitis indices or CRP values on the other. Several reasons could potentially explain this. First, the cross-sectional study design does not permit a good estimate of the cumulative inflammatory burden. Second, all patients included in this study had high disease activity, which may have diminished the potential to observe such an association. However, this argument does not apply to CRP values, which varied widely among AS patients. Hence, the inflammatory hypothesis may perhaps apply to AS to a lesser extent than to these other rheumatic diseases. That noted, CRP values, although several times higher than in controls, are considerably lower in AS patients than in other rheumatic diseases, such as rheumatoid arthritis and psoriatic arthritis. Third, somewhat paradoxically, despite stringent inclusion criteria used in this study, clinical heterogeneity of patients may well have been too large. For instance, patients with active disease and high BASMI values may have suffered from limited spinal mobility due to pain/inflammation rather than advanced radiological damage (35). In all, the lack of an association between inflammatory mediators and arterial wall properties does not per se exclude the possibility that the chronic inflammatory burden is atherogenic, and further studies are needed to address this association.

The results presented here suggest that an adverse CV risk profile causes, at least in part, the greater IMT and higher pulse pressure in AS patients. In fact, body mass index, total cholesterol and triglycerides were positively correlated with IMT and/or arterial stiffness (7;29). In conjunction with this, 23 of the consecutive 82 screened AS patients (28%) were excluded from analyses, because of prior CV disease or other important CV risk factors. These observations are in line with the findings of others (26;29), and may imply that CV risk factors have more impact on the CV system than AS itself, and underline the importance of proper CV management (36).

Interestingly, despite a higher carotid pulse pressure in AS, carotid vessel wall properties did not differ between AS and control subjects. Apparently, other segments of the arterial tree are responsible for the increased pulse pressure, although a role for increased stroke volume/contractility cannot be fully excluded. An impaired aortic compliance may be responsible for the higher pulse pressure as this is the arterial segment that predominantly affects pulse pressure throughout the arterial tree (37). However, to date, no studies have been published on aortic compliance in AS, and our findings illustrate the necessity to perform such studies. Whatever the cause, our observations are important as a higher pulse pressure itself translates into higher CV risk (38).

Interpretation of our findings needs to be done with caution as all observations are cross-sectional and do not provide definitive evidence for causality or directionality. In addition, a

single-point measurement technique was used to determine arterial properties, i.e. cIMT, diameter and distension, and this may have influenced our results due to variability of these characteristics along the arterial segment. However, we minimized measurement variability by clearly defining where IMT had to be determined. Some patients had obvious AS specific deformities and this made blinding impossible. Furthermore, it should be noted that a large proportion (~50%) of the AS patients smoked, which may have caused bias towards the null hypothesis. However, multivariable adjustment should have accounted for such bias tendency. Moreover, when patients and controls that smoked were excluded from the comparison analysis, the age- and gender matched IMT difference remained. In agreement, all correlations between arterial wall properties and risk factors remained when patients that smoked were excluded. All AS patients were treated with stable doses of NSAIDs and this may be a potential confounder explaining the observed differences in IMT and arterial stiffness (39). To disentangle the CV effect of inflammation and NSAIDs, one may compare patients with non-inflammatory back pain using NSAID with AS patients. The fact that controls were having a significantly lower heart rate, which may reflect more physical activity and, were less smoking, may indicate that this group was more vigilant about their cardiovascular health. Finally, since AS patients included in this study were highly selected, we cannot necessarily conclude that AS patients with a lower disease activity do have similar changes in arterial wall properties as observed in the present study.

Taken together, our observations indicate that AS is associated with early signs of preclinical atherosclerosis, but future larger (prospective) studies are needed to confirm our findings and to investigate which factors contribute to these arterial perturbations.

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Chapter 1.2

Microvascular Function is Impaired in Ankylosing Spondylitis and Improves after TNF α Blockade

I.C. van Eijk
M.J.L. Peters
E.H. Serné
I.E. van der Horst-Bruinsma
B.A.C. Dijkmans
Y.M. Smulders
M.T. Nurmohamed

ABSTRACT

Objective. Ankylosing spondylitis (AS) is associated with increased cardiovascular morbidity and mortality. Microvascular function has been linked to several risk factors for cardiovascular disease. Inflammation in AS may cause microvascular dysfunction. To test this, we assessed microvascular function in 1] AS patients compared to healthy controls and 2] AS patients before and after one month of anti-TNF α treatment with etanercept.

Methods. Fifteen consecutive AS patients, who were scheduled for etanercept treatment according to the ASAS guidelines, and 12 healthy controls matched for age and sex, were recruited. Endothelium-dependent and –independent vasodilatation in skin were evaluated with laser Doppler fluxmetry after iontophoresis of acetylcholine and sodium nitroprusside, respectively. Videomicroscopy was used to measure recruitment of skin capillaries after arterial occlusion.

Results. Compared to healthy controls, AS patients had impaired endothelium-dependent vasodilatation and capillary recruitment. Following anti-TNF α treatment, microvascular function improved significantly for both endothelium-dependent vasodilatation ($P=0.03$) and capillary recruitment ($P=0.006$). A significant correlation was observed between changes in endothelium-dependent vasodilatation and changes in ESR ($r=-0.56$; $P=0.03$).

Conclusions. Microvascular dysfunction is present in AS patients, with active disease, but improves as inflammation regresses after TNF α blockade.

INTRODUCTION

AS is a chronic inflammatory disease of the sacroiliac joints and spine affecting up to 1 % of the population (1). Patients with AS have an approximately twofold increased mortality rate compared to the general population, which is predominately caused by increased cardiovascular (CV) risk (2-4). Although specific cardiovascular disorders (valvular disease and conduction disturbances) occur more frequently in AS (4;5), accelerated atherosclerotic disease probably contributes to the increased CV risk as well (6;7). Accelerated atherosclerosis in AS may partly be due to traditional CV risk factors, i.e. an atherogenic lipid profile and hypertension (8;9). In addition, the generalised inflammatory state that characterises active AS renders these patients more prone to develop cardiovascular, atherosclerotic, disease (CVD), as many parallels exist between the inflammatory mechanisms in the pathogenesis of atherosclerosis and in the pathogenesis of auto-immune diseases (10). Therefore, AS could be an independent cardiovascular risk factor (6;7). The association between inflammation and atherogenesis is most extensively described for rheumatoid arthritis (RA) and systemic lupus erythematosus (11-14) but appears to apply to AS as well (6;7).

Analogous to RA (15), inflammation in AS may cause (microvascular) endothelial dysfunction. Endothelial dysfunction precedes and initiates atherosclerosis and is a predictor of long-term cardiovascular risk (16). A pivotal pro-inflammatory cytokine is tumour necrosis factor alpha ($\text{TNF}\alpha$). Circulating $\text{TNF}\alpha$ levels are increased in AS patients and important in the pathogenesis of AS (17;18). Increased $\text{TNF}\alpha$ levels may have an important role in the pathogenesis of endothelial dysfunction in inflammatory diseases (19-21).

A particularly interesting type of vascular dysfunction occurs in the microvasculature. Microvascular dysfunction is closely, and presumably causally, linked to CV risk factors, particularly insulin resistance and hypertension (22;23). Preliminary evidence suggests that inflammation may also cause dysfunction of the microvasculature. Impaired coronary microvascular function was recently found in AS patients, and correlated well with serum C-reactive protein (CRP) and $\text{TNF}\alpha$ levels (19). Interestingly, circulating levels of CRP and $\text{TNF}\alpha$ are associated with skin microvascular dysfunction even in normal subjects (24;25).

Considering this, we hypothesize that patients with active AS have impaired microvascular function. In addition, we anticipate that blocking $\text{TNF}\alpha$ in these patients not only reduces disease activity (26-29), but also improves microvascular function. To test this, we assessed capillary density and recruitment as well as endothelium-(in)dependent vasodilatation of skin microcirculation in 1] AS patients compared to healthy controls and 2] AS patients before and after one month of anti- $\text{TNF}\alpha$ treatment with etanercept.

PATIENTS AND METHODS

Subjects

Fifteen consecutive AS patients (10 males), scheduled for etanercept treatment according to the ASAS guidelines for anti-TNF α treatment (30) were studied. All patients fulfilled the modified New York diagnostic criteria of AS (31) and were recruited from the Jan van Breemen Institute and VU University Medical Center and treated with twice weekly 25 mg etanercept subcutaneously. Exclusion criteria were (self reported) diabetes mellitus, Raynaud phenomenon, thyroid dysfunction, previous cardiovascular events, hypertension (systolic blood pressure > 140 mmHg and/or diastolic blood pressure > 90 mmHg) or the use of antihypertensive agents. Twelve age- and sex-matched healthy volunteers (8 males) served as controls. One patient and one control subject used a statin. No alterations in (concomitant) medication use, including non steroidal anti-inflammatory drugs, which all patients used, were allowed during the treatment period. All participants gave written informed consent and the study protocol was approved by the Institutional Ethics Committee of both hospitals.

Study Design

Microvascular measurements were conducted in a quiet, temperature-controlled room ($T=23.4\pm0.4^{\circ}\text{C}$) after 20-30 minutes of acclimatization, with the subjects in the sitting position and the investigated, non-dominant hand at heart level. Nailfold capillary studies and iontophoresis studies were performed on the same day. Subjects were asked to refrain from beverages other than water (especially no caffeine or alcohol), smoking, medication, except paracetamol if necessary, and meals from midnight at the testing day. During the tests, skin temperature was monitored and all subjects were studied between 8.00 am and 12.00 am.

Nailfold capillaries in the dorsal skin of the third finger were visualized by a capillary microscope (Nikon), linked to a television camera (Philips LDH 0702/20), a video recorder (Panasonic NV-HS930, S-VHS) and a monitor (Sony SSM-125CE) (32). Incident illumination was achieved by light from a 100-W vapor mercury lamp, which passes through a heat-absorption and heat-reflection filter, a polarizer, and a 50% mirror to illuminate the object. To visualize the capillaries, a 5x objective (Nikon 5/0.13) was used with a total system magnification of 115x. Nailfold capillaries in finger skin were recorded on videotape before and after 4 minutes of arterial occlusion with a digital cuff. This procedure was performed twice, and the mean of both measurements was used for analyses. Counting was performed using monitor (Sony PVM-1443MD) and video recorder (Sony S-VO-9500MDP). Capillaries were counted by a single observer using the naked eye from a freeze-framed reproduction of the videotape and from the running videotape, when it was uncertain whether a capillary was

present or not. We estimated baseline capillary density by counting the number of continuously erythrocyte-perfused capillaries during a 15-second period. Other capillaries can be seen to be intermittently perfused, and these may represent an important functional reserve. We used post-occlusive reactive hyperemia to estimate this functional reserve. Post-occlusive capillary recruitment was calculated by dividing the increase in density by the baseline density. The day-to-day coefficient of variation (CV) of the capillary density in resting state was $2.3 \pm 1.8\%$. The CV of the percentage capillary recruitment and absolute capillary recruitment during post-occlusive hyperaemia were $8.3 \pm 4.9\%$ and $6.2 \pm 4.3\%$, respectively.

Endothelium-(in)dependent vasodilatation of finger skin microcirculation was evaluated by iontophoresis of acetylcholine (ACh) and sodium nitroprusside in combination with laser Doppler fluxmetry as previously described in more detail (22;33). Laser Doppler fluxmetry measures microvascular perfusion, the product of red blood cell velocity and concentration (34). A protocol of multiple fixed doses (current intensity x delivery time) was employed resulting in an incremental dose-response curve. Acetylcholine (1%, Miochol-E, Théa Pharma NV) was delivered with an anodal current; 7 doses (0.1 mA for 20 seconds) were delivered, with a 60-second interval between each dose. A 60-second interval between each iontophoresis period was required to achieve the plateau of the response following each delivery of acetylcholine (35). Sodium nitroprusside (0.01%, VUmc) was delivered with a cathodal current; 9 doses (0.2 mA for 20 seconds) were delivered, with a 90-second interval between each dose. A 90-second interval between each iontophoresis period was required to achieve the plateau of the response after each delivery of sodium nitroprusside (35). Acetylcholine-dependent laser Doppler flux was measured on the middle phalanx of the third finger, whereas nitroprusside-dependent laser Doppler flux was measured at the same spot on the opposite hand, with approximately 15 minutes elapsed between the two measurements. The day-to-day CV of the percentage increase from baseline to the final two minutes of the plateau phase was $9.8 \pm 5.6\%$ for acetylcholine and $8.3 \pm 5.4\%$ for sodium nitroprusside.

Statistical Analyses

Data are expressed as mean (SD) or median (interquartile range) as appropriate. The distribution of variables was tested for normality and transformed if necessary. Variables were tested 1) unpaired for comparing patients and controls and 2) paired for comparing the AS patients at baseline and at one month. Student's t-test was used to compare continuous normally distributed variables. We used nonparametric tests (Wilcoxon signed-rank test or Mann Whitney U-test) when appropriate. Linear regression analysis was used to investigate confounding by body mass index (BMI, as calculated using the formula weight (kilogram) /

length (metres)² * 100). A two-tailed probability value of $P < 0.05$ was considered statistically significant. Correlations between variables were analysed by using Pearson correlation or Spearman's rho tests when appropriate.

RESULTS

Characteristics

Baseline characteristics of patients and controls are shown in table 1 and 2. Age, sex, blood pressure and smoking status were similar in both groups. BMI was higher in patients compared to controls, 26.9 [24.2-28.4] versus 23.2 [22.2 – 24.9] respectively ($P=0.06$). Within patients, BMI and blood pressure remained stable between the two time points. During treatment, CRP and ESR levels, as well as BASDAI decreased significantly.

Table 1. Baseline characteristics of the AS patients and healthy control subjects

	AS (n=15)	Controls (n=12)
Age, years	40 ± 10	40 ± 13
Male, %	73	67
Disease duration, years	10.8 ± 8.3	
Smoking, %	47	42
Systolic blood pressure, mmHg	122 ± 8	123 ± 11
Diastolic blood pressure, mmHg	77 ± 5	77 ± 5
Pulse pressure, mmHg	44 ± 7	46 ± 9
BMI	26.9 (24.2 – 28.2)	23.2 (22.2 – 24.9)

Data are mean +/-SD or median (Interquartile range); Student's T-test or Mann-Whitney U test were used for comparing continuous variables. Fisher's exact test was used for dichotomous variables. Pulse pressure = systolic blood pressure – diastolic blood pressure.

Microvascular function is disturbed in AS

At baseline, AS patients showed a markedly lower vasodilatation in response to acetylcholine compared to controls (118% versus 469%, respectively, $P=0.02$; table 3 and fig 1). The response to sodium nitroprusside did not differ significantly between patients at baseline and one month and controls.

Baseline capillary density did not differ between groups. However, absolute and relative post-ischemic capillary recruitment were lower in AS at baseline compared to controls (14.8 versus 19.5, respectively for absolute increase, $P=0.04$ and 29.6% versus 39.2%, respectively for relative increase, $P=0.01$). These results remained after separately adjusting for the difference in BMI and the difference in smoking status between the two groups (data not shown).

Table 2. Characteristics of the Ankylosing Spondylitis patients at baseline (AS pre) and after 1 month etanercept treatment (AS post)

	AS pre (n=15)	AS post (n=15)	p
ESR, mm/h	19 (5 – 45)	4 (1 – 13)	0.001
CRP, mg/dl	14 (4 – 37)	3 (1 – 8)	0.006
BASDAI	6.0 (5.3 – 7.2)	4.4 (1.8 – 6.4)	0.004
BASFI	5.2 (4.0 – 6.3)	3.2 (1.7 – 5.1)	0.003
BASMI	3.0 (2.0 – 4.0)	2.0 (2.0 – 4.0)	0.3
Total Cholesterol, mmol/l	5.0 ± 0.7	5.3 ± 0.8	0.04
HDL, mmol/l	1.3 ± 0.4	1.4 ± 0.3	0.05
LDL, mmol/l	3.1 ± 0.7	3.2 ± 0.7	0.6
TG, mmol/l	1.3 0.7±	1.6 ± 1.0	0.3

Data are mean +/-SD or median (Interquartile range). HDL = high density lipoprotein; LDL = low density lipoprotein; TG = triglycerides. Lipid profile was available for 13 patients (non-fasting).

Table 3. Microvascular measurements

	Controls (n=12)	AS pre (n=15)	AS post (n=15)	P (1)	P (2)	P (3)
ACh-mediated vasodilatation						
Skintemperature, °C	30.6 ± 1.3	30.6 ± 1.0	31.0 ± 1.4	0.8	0.4	0.4
Baseline skin perfusion, PU	21.9 ± 10.8	21.9 ± 7.5	24.3 ± 11.5	1.0	0.4	1.0
ACh-mediated vasodilatation, %	469 (174 – 692)	118 (43 – 288)	318 (37 – 591)	0.02	0.03	0.5
SNP-mediated vasodilatation						
Skintemperature, °C	30.2 ± 1.1	30.4 ± 1.0	31.5 ± 2.6	0.7	0.2	0.2
Baseline skin perfusion, PU	17.8 ± 10.5	18.7 ± 7.8	23.1 ± 9.2	0.7	0.07	0.2
SNP-mediated vasodilatation, %	391 (152 – 1116)	348 (81 – 649)	200 (73 – 401)	0.1	0.3	0.05
Capillary recruitment						
Skintemperature, °C	30.3 ± 0.9	30.4 ± 1.4	30.7 ± 1.5	1.0	0.2	0.4
Baseline capillary density, number/mm ²	49.0 ± 6.9	50.6 ± 13.4	53.3 ± 11.0	0.7	0.1	0.3
Peak capillary density, number/mm ²	68.5 ± 12.7	65.4 ± 16.8	73.9 ± 15.9	0.6	0.003	0.4
Absolute increase, number/mm ²	19.5 ± 6.5	14.8 ± 5.2	20.7 ± 6.7	0.04	0.001	0.7
Capillary recruitment, %	39.2 ± 9.0	29.6 ± 9.3	38.9 ± 10.9	0.01	0.006	1.0

Data are mean +/-SD or median (Interquartile range); P values were calculated: (1) AS pre versus controls; (2) AS pre versus AS post; (3) AS post versus controls. ACh = acetylcholine; SNP = sodium nitroprusside; PU = perfusion units

Etanercept improves microvascular dysfunction

After one month of treatment with etanercept, endothelium-dependent vasodilatation improved significantly in the AS patients (from 118% to 318%, $P=0.03$; table 3 and fig 1) and approached values observed in the healthy controls ($P=0.5$). Capillary recruitment improved significantly in AS patients during treatment (from 29.6% to 38.9%, $P=0.006$; table 3) to a level comparable to controls (38,9% versus 39,2%, respectively, $P=1.0$).

Etanercept-induced changes in endothelium-dependent vasodilatation correlated inversely with changes in ESR levels ($r=-0.56$ $P=0.03$, fig 2). We did not find significant correlations between pre- and post-treatment differences in endothelium-dependent vasodilatation relative to CRP ($r=-0.24$ $P=0.4$, fig 2) or BASDAI ($r=-0.16$ $P=0.6$). Changes in capillary recruitment (percentage) were not significantly correlated with changes in ESR, CRP or BASDAI ($r=-0.15$ $P=0.6$, $r=0.014$ $P=1.0$, $r=0.50$ $P=0.06$ respectively), although there was a trend for BASDAI.

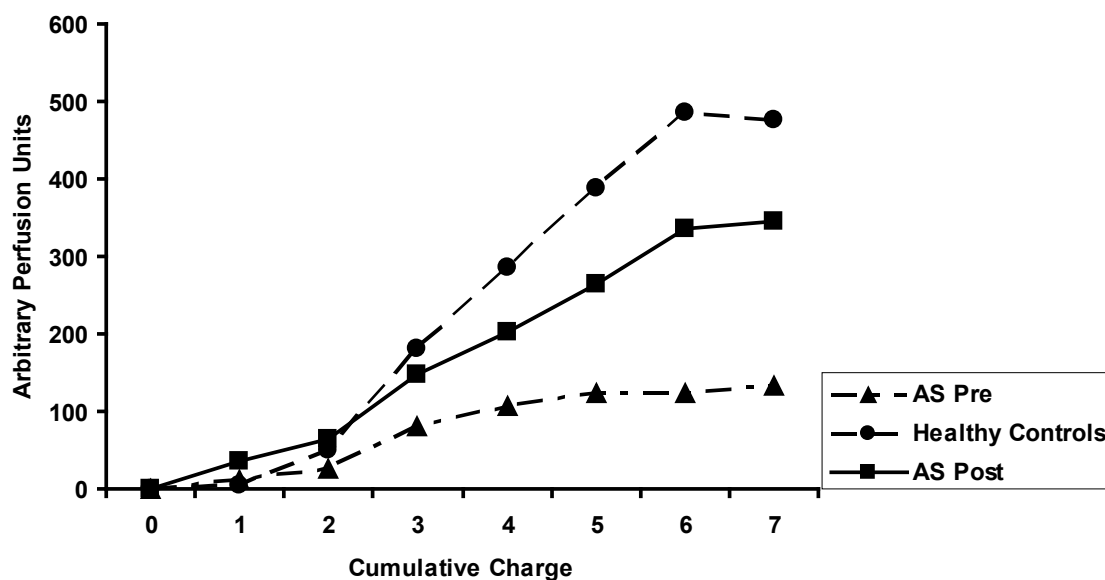


Figure 1. Perfusion changes in response to iontophoresis of acetylcholine in the AS patients pre- and post-treatment and in the healthy control subjects. Baseline perfusion was set to 0% and every subsequent dot represents the percentual perfusion change compared to baseline. Every dot represents the median perfusion value of the 60 seconds between two subsequent pulses as described in the methods section.

DISCUSSION

The present study demonstrates that patients with active AS have impaired microvascular endothelium-dependent vasodilatation and capillary recruitment in skin, which improves after TNF α blocking therapy with etanercept.

Recent advances have highlighted the crucial involvement of the microcirculation in many cardiovascular conditions, not only in the development of target-organ damage in the heart and kidney, but also in the development of cardiovascular risk factors such as hypertension and insulin resistance (23;36;37). Impaired microvascular endothelium-dependent vasodilatation and capillary recruitment, as measured in the present study, have been linked to several conditions associated with CVD, such as hypertension, insulin resistance and (visceral) obesity (22;23;25;33). Furthermore, impaired endothelium-dependent vasodilatation and capillary recruitment can be detected in individuals at increased coronary heart disease (CHD) risk according to the Framingham Heart Study risk score (38).

The cutaneous microcirculation is a representative vascular bed to examine the mechanisms of microvascular dysfunction, which may mirror generalized systemic vascular dysfunction in magnitude and underlying mechanisms (39). The finding of impaired microvascular function in AS patients is concordant with a previous study demonstrating impaired coronary microvascular function in AS patients without any overt cardiovascular disease (19). Interestingly, in the latter study, impaired coronary microvascular function correlated well with CRP and TNF α levels, suggesting a detrimental effect of inflammation on the microcirculation (19). Our findings support this hypothesis by showing, for the first time, that blocking TNF α with etanercept, results in improvement of microvascular endothelium-dependent vasodilatation and even a normalization of capillary recruitment in AS patients compared to healthy controls. In RA patients, the ability of TNF α blocking therapies to improve endothelial function has already been demonstrated, predominately on macrovascular level, as measured by brachial artery ultrasonography (20;21) or plethysmography (40). Again in RA patients, a case of diffusely impaired myocardial perfusion in the absence of any significant coronary atheroma in active systemic RA was described, which improved following intensive immunosuppression (41). Moreover, impaired endothelium-dependent vasodilatation has been shown at the microvascular level during active disease ("flare"), which improved after inflammatory suppression with disease modifying anti-rheumatic drugs (DMARDs) or TNF α blocking therapy (42).

In this regard, the observation of a lower incidence of first cardiovascular events in RA patients treated with TNF α blocking therapy (43) is interesting, as improvement of microvascular function after TNF α blockade may play a causative role.

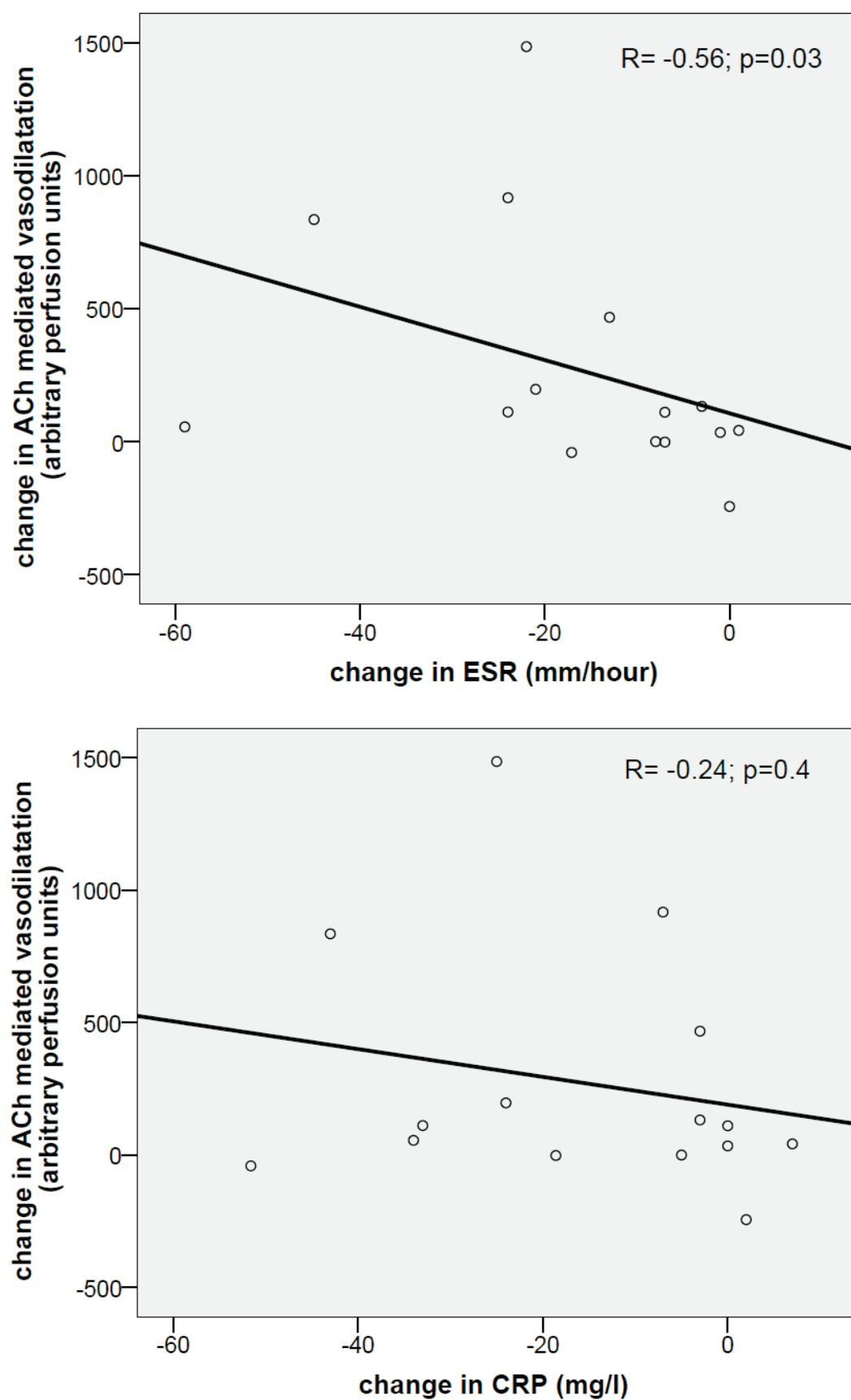


Figure 2. Correlation between changes in acetylcholine mediated vasodilatation (post - pre treatment, relative values) and changes in ESR and CRP levels

There is evidence that $\text{TNF}\alpha$ contributes to microvascular dysfunction. $\text{TNF}\alpha$ levels negatively correlate with skin capillary recruitment even in healthy individuals (24;25) and increased production of $\text{TNF}\alpha$ is associated with obesity-related insulin resistance, as well as obesity-related hypertension (25;44). A possible mechanism involved in these microvascular disturbances is impaired activation of endothelial nitric oxide synthase (eNOS). $\text{TNF}\alpha$ blocks the activation of eNOS, by interfering with Akt phosphorylation, which is essential for flow-dependent and ACh-dependent vasodilatation (45). In addition, $\text{TNF}\alpha$ directly degrades eNOS mRNA (46;47).

In addition to specific $\text{TNF}\alpha$ effects on microvasculature, it is conceivable that, in line with inflammatory driven atherogenesis, improvement of microvascular function was the result of a generally decreased inflammatory “burden”. Indeed, ESR and CRP levels, as markers of inflammation, declined significantly during treatment. In this regard, in 50 psoriatic arthritis patients, another inflammatory disease included within the group of spondyloarthropathies, without clinically evident cardiovascular disease, a significant correlation between CRP level and ESR at the time of disease diagnosis and flow-mediated endothelial dependent vasodilatation was observed (48). Correlation analyses revealed a significant correlation for pre- and after treatment ESR levels and endothelium-dependent vasodilatation, but not for CRP. The absence of a significant correlation between changes in CRP and vascular function was also reported in previous studies on vascular function in RA and AS (42;49). In our study, this may be due to small patient numbers, or indicate that, in addition to inflammatory suppression, $\text{TNF}\alpha$ specific features determining endothelial function, are important. The latter is supported by a study showing improvement of endothelial function in RA patients after intravascular administration of a $\text{TNF}\alpha$ blocking agent, without concurrently affecting circulating CRP levels (50). Probably both mechanisms i.e. general dampening of inflammation and (other) $\text{TNF}\alpha$ specific effects intertwine. The present study design, however, is not suited to clearly differentiate between these mechanisms.

In conclusion, we showed that patients with active AS have vascular dysfunction at the microcirculatory level. This finding supports the idea that AS represents a CHD risk factor. $\text{TNF}\alpha$ blocking therapy with etanercept improved microvascular function. Further research in larger patient groups should elucidate if this effect translates into a decrease in cardiovascular morbidity and mortality in AS patients.

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Chapter 1.3

Improvement of lipid profile is accompanied by atheroprotective alterations in high density lipoprotein composition upon TNF blockade

A prospective cohort study in Ankylosing Spondylitis

I.C. van Eijk
M.K. de Vries
J.H.M. Levels
M.J.L. Peters
E.E. Huizer
B.A.C. Dijkmans
I.E. van der Horst-Bruinsma
B.P.C. Hazenberg
R.J. van de Stadt
G.J. Wolbink
M.T. Nurmohamed

ABSTRACT

Objectives. Cardiovascular mortality is increased in ankylosing spondylitis (AS) and inflammation plays an important role. Inflammation deteriorates lipid profile and alters high density lipoprotein (HDL-c) composition reflected by increased concentrations of serum amyloid A (SAA) within the particle. Anti-TNF treatment may improve these parameters. Hence, we investigated the effects of etanercept on lipid profile and HDL composition in AS.

Methods. In 92 AS patients, lipid levels and their association with inflammatory markers CRP, ESR and SAA were evaluated over time during 3 months etanercept treatment. HDL composition and its relationship with inflammatory markers was determined in a subgroup using Surface-enhanced-laser desorption/ionization-time-of-flight analysis.

Results. Upon anti-TNF treatment, all inflammatory parameters decreased significantly, whereas total cholesterol (TC), HDL-c and apolipoprotein A1 (apoA-1) significantly increased. This resulted in a better TC/HDL-c ratio (from 3.9 to 3.7), albeit not statistically significant, and an improved apoB/apoA-1 ratio, which decreased 7.5% over time ($p<0.01$). In general, increases in levels of all lipid parameters were associated with reductions in inflammatory activity. In addition, SAA was highly present within HDL particles of AS patients with increased CRP levels, and disappeared during treatment, along with declining plasma levels of SAA.

Conclusions. We showed for the first time that during anti-TNF treatment for AS, along with favourable changes in lipid profile, HDL composition is actually altered, with SAA disappearing from the HDL particle, rendering it more atheroprotective. These findings underline the importance of understanding the role of functional characteristics of HDL cholesterol in cardiovascular diseases, related to chronic inflammatory conditions.

INTRODUCTION

Ankylosing spondylitis (AS) is a chronic inflammatory disease of the sacroiliac joints and spine affecting up to 1% of the population (1). Patients with AS have an approximately twofold increased mortality rate compared to the general population, which is predominately caused by increased cardiovascular (CV) risk (2-4). Although specific CV disorders (valvular disease and conduction disturbances) occur more frequently in AS (4;5), accelerated atherosclerotic disease probably contributes to the increased CV risk as well (6;7). Despite the fact that atherosclerosis is a multifactorial process, the most established risk factor for atherosclerosis is dyslipidemia. Important prognostic indicators for CV disease are the ratio of total cholesterol (TC) to high density lipoprotein cholesterol (HDL-c) (TC/HDL-c) and the apolipoprotein B/ apolipoprotein A-1 (apo-B/apoA-1) ratio. Inflammation deteriorates the lipid profile, characterized by low HDL, TC and apoA-1 levels and increased levels of low density lipoprotein cholesterol (LDL-c), triglycerides (TG) and apoB. Indeed, several investigations have reported that patients with inflammatory rheumatic diseases have a deteriorated lipid profile (4;6;8-10).

In addition to changes in lipid levels, inflammation can affect HDL qualitatively (11). During inflammation specific enzyme and protein components of HDL, contributing to HDL's (anti)atherogenic potential such as serum amyloid A protein (SAA) and apoA-1, are modified and may even render it proatherogenic (12).

Tumor necrosis factor alpha (TNF α) is a pivotal pro-inflammatory cytokine in inflammatory diseases and causes a deterioration of the lipid profile in inflammatory conditions (13). Treatment with TNF blocking agents, in addition to their known powerful anti-inflammatory effects, may therefore have a beneficial effect on the lipid profile as well as on HDL composition (14;15).

The current study was designed to investigate whether modulating inflammatory activity by TNF blocking therapy in AS patients with active disease, is associated with alterations in lipid profile and qualitative changes in HDL composition

MATERIALS AND METHODS

Patients

Consecutive AS patients attending the outpatient clinics of the Jan van Breemen Institute and VU University medical center in whom etanercept treatment was initiated according to the ASAS consensus statement for initiation of anti-TNF treatment (16), were included and followed prospectively. All patients fulfilled the 1984 modified New York criteria and were

treated with subcutaneous etanercept 25 mg twice-weekly or 50 mg once weekly. A high disease activity was defined as a BASDAI ≥ 4 . Patients were included if a baseline serum sample and at least one follow up serum sample were available. The study was approved by the medical ethical committee and all patients gave written informed consent.

Study Design

Data were collected at baseline, after 1 month and 3 months. During every visit questionnaires on disease activity (BASDAI) were obtained. TC, HDL-c, LDL-c, triglycerides, apoA-1, apo B, SAA, Erythrocyte Sedimentation Rate (ESR) and C-reactive protein (CRP) were measured in patients' sera at all time points. Collected sera were stored at -20°C until testing. Commercially available kits were used to measure these acute phase reactants.

Laboratory measurements

Assessment of lipids

Serum total cholesterol and triglycerides, were analyzed by an enzymatic method using the appropriate assays supplied by Roche Diagnostics (Almere, The Netherlands), on a Cobas 6000 analyzer (Roche) according to the instructions of the manufacturer. PEG-modified enzymes were used for assessing the HDL-c levels. ApoA-1 and apoB were analyzed by an immunoturbidimetric method, using the appropriate assays supplied by Roche Diagnostics.

Since we were not able to directly measure LDL-c levels in our laboratory, the Friedewald formula was used, on condition that triglycerides levels were lower than 400 mg/dL, to calculate LDL-c levels, although, in the strictest sense, this formula may not be the most appropriate method to determine LDL-c in nonfasting samples. The TC/HDL-c ratio was calculated as the TC level divided by the HDL-c level.

Assessment of inflammatory markers

CRP levels were determined using the Roche/Hitachi cobas c analyzer, based on the principle of particle-enhanced immunological agglutination (Roche Diagnostics GmbH, D-68298, Mannheim). Values are expressed in milligrams/litre. A CRP below 10 mg/L was considered to be normal. High sensitivity CRP (hsCRP) levels were determined using the Roche/Hitachi cobas c systems with a detection range of 0.15-20 mg/L. The test principle consists of a particle enhanced immuno-turbidimetric assay. Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies.

ESR was determined with local measurement techniques (Westergren method) and expressed in millimeters per hour (N men <20 mm/hr, women <30 mm/hr).

SAA was assessed with an enzyme-linked immunosorbent assay (ELISA) as described previously (17). A value below 4 mg/L was considered to be normal.

Sample preparation

HDL protein profiling was carried out as described previously (18). For coating of antibody, a 5 ml mixture containing 2.8 nM anti-apoA-I monoclonal antibodies, 3 mM ethylenediamine, and 0.1 M Na₂SO₄ was added per spot of a PS-20 protein chip, and covalent binding of antibodies through primary amine-epoxide chemistry was achieved by incubating the chip in a humid chamber overnight at 4 °C. Excess antibody was removed by one wash with distilled water, and subsequently, free amine binding places were blocked by incubating the chip for 30 min at room temperature with 1 M Tris buffer (pH 8.4). For HDL capture, after mounting the PS-20 protein chip(s) in a 96-well bioprocessor, 100 µl of diluted plasma aliquots (diluted 1:2 with Tris Buffered Saline (TBS) buffer: 50 mM Tris, pH 7.4, and 150 mM NaCl) was applied onto each spot and allowed to bind for 2 hours at room temperature on a horizontal shaker. The protein chips were washed four times with TBS for 5 min, followed by a 2 min TBS-Tween (0.005%) rinse unless indicated otherwise. A final wash step with HEPES solution (5 mM) was carried out to remove the excess salt. All spots were allowed to dry, and subsequently, 1.2 µl of sinapinic acid (10 mg/ml) in a 50:49.9:0.1% acetonitrile-water:trifluoroacetic acid mix was applied on each spot. All chips were airdried and stored at room temperature in the dark until analysis. These measurements were carried out at the same day as the chip processing.

Surface-enhanced laser desorption/ionization time-of-flight analysis (SELDI-TOF)

Analysis was carried out using a PBS IIc protein chip reader (Ciphergen Biosystems, Fremont, CA) using an automated data collection protocol within the Protein-Chip Software (version 3.1). Data were collected up to 200 kDa. Laser intensity was set in range from 190 to 200 arbitrary units at a sensitivity of 7, and the focus mass was set to 28 kDa specific for the anti-apo A-I capture. Measurement of the spectra was performed with an average of 100 shots at 13 positions per SELDI spot. Calibration was done using a protein calibration chip (Ciphergen). Spectra were normalized on total ion current. Detected peaks having a signal-to-noise ratio of 5 were recognized as significant peaks. For data on the reproducibility of the Seldi technique see (18).

Statistical analysis

Data are expressed as mean (SD) or median (interquartile range) as appropriate. The distribution of variables was tested for normality and transformed if necessary. Independent t-tests were used for variables with a normal distribution and nonparametric tests (Wilcoxon signed-rank test or Mann Whitney U-test) for skewed variables. Pearson chi square tests were done for dichotomous variables. Correlation coefficients (Pearson) were calculated to evaluate correlations between SAA and lipid levels at baseline.

The generalized estimating equations (GEE) approach was used; 1) to analyze the longitudinal data of the lipids, lipoproteins and acute phase reactants, measured at three different time points (in other words, a longitudinal logistic regression analysis was performed) and 2) to investigate associations between changes in disease activity markers, HDL-c and apoA-1 levels over time. Absolute and relative changes of lipids were calculated for changes in disease activity parameters. As the TC/HDL-c ratio and triglyceride levels were not normally distributed, data were analysed with the logarithms of these values. For clarity, the regression coefficients for these lipids were retransformed to geometric means. Calculations were made using SPSS 16.0 software. The threshold for significance was set at $p < 0.05$.

RESULTS

Characteristics

A total of 92 consecutive AS patients (60 male (65%)) were enrolled. Median age was 40.6 years, with a mean BASDAI of 6.0 and median disease duration of 9 years. Ninety-four percent of the patients used NSAIDs, 22% used concomitant disease modifying anti-rheumatic drugs and 8% was known to use statins. During treatment, all pharmacological treatment remained unchanged. Baseline characteristics are shown in table 1.

Inflammatory markers

Concentrations of inflammatory parameters ESR and CRP and HsCRP were elevated at baseline and decreased during treatment ($p < 0.001$) (table 2). The same was true for SAA, another acute-phase protein, with elevated levels at baseline that decreased significantly after 1 month and remained stable and low thereafter ($p < 0.001$) (table 2). At baseline SAA correlated negatively with apoA-1 levels ($r = -0.28$ $p = 0.08$), indicating that higher plasma SAA levels were accompanied by lower apoA-1 levels. Baseline SAA levels were not correlated with HDL-c ($r = 0.07$ $p = 0.5$).

Lipid levels over time

Table 2 shows lipid levels and disease activity parameters in AS patients before and during anti-TNF treatment. TC, HDL-c and apoA-1 levels increased significantly during treatment ($p < 0.001$, $p < 0.001$ and $p = 0.004$ respectively). LDL-c and TG slightly increased during treatment ($p = 0.04$ and $p = 0.03$ respectively), and apoB remained stable. The TC/HDL-c ratio decreased from 3.9 at baseline to 3.7 (5%) after 3 months, but this did not reach statistical significance. The apo-B/apoA-1 ratio decreased with 7.5% from 0.67 to 0.62 ($p = 0.008$).

Table 1. Baseline characteristics of the AS patients

Demographic features	
N	92
Age (years)	43 (11.2)
Disease duration (years)	8.5 (3-18)
Male/ female	60/32
HLA-B27 positive (number, %)	74 (88)
Disease activity parameters	
ESR (mm/hr)	21 (6 - 38)
CRP (mg/l)	13 (3 - 35)
HsCRP (mg/l)	11.3 (3.1 – 33.2)
SAA (mg/l)	5 (2-18)
BASDAI (0-10)	6.0 (1.5)
Lipids	
TC (mmol/l)	4.87 (0.9)
HDL-c (mmol/l)	1.29 (0.4)
TC/HDL-c	3.89 (3.01 – 4.90)
LDL-c (mmol/l)	2.92 (0.8)
Triglycerides (mmol/l)	1.17 (0.89 – 1.74)
apoA-1 (g/l)	1.39 (0.3)
apoB (g/l)	0.88 (0.2)
apoB/apoA-1 ratio	0.67 (0.23)

AS, Ankylosing Spondylitis; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index 0-10 scale; ESR, erythrocyte sedimentation rate; CRP, C reactive protein; HsCRP (high sensitivity CRP); TC, SAA, serum amyloid A; total cholesterol; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; ApoA-1, apolipoprotein A1; apoB, apolipoprotein B; atherogenic index, ratio of TC/HDL. Values are mean (SD) or median (interquartile range), as applicable.

Associations between lipids and disease activity markers

Since CRP and HsCRP levels were comparable only CRP was used in the association models. GEE analyses showed several significant associations between lipid levels and the disease activity parameters including CRP, ESR, SAA and BASDAI over time. In other words, the height of the disease activity parameters influenced the height of the lipid levels significantly. Table 3 shows the influence of disease activity parameters on lipid levels. During the 3 month follow up period, decreasing levels of CRP, ESR, SAA and BASDAI levels were significantly associated with increasing TC levels ($p \leq 0.003$) (with regression coefficients of 0.01, 0.015, 0.006 and 0.063 respectively), with increasing HDL-c levels ($p \leq 0.014$) (with regression coefficients of 0.004, 0.005, 0.002, 0.025 respectively), with increasing apoA-1 levels ($p \leq 0.001$) (with regression coefficients of 0.004, 0.005, 0.003, 0.018 respectively) and with a decreased ApoB/ApoA-1 ratio ($p < 0.01$) (with regression coefficients

of -0.002, -0.002, -0.001, -0.001 respectively). Changes in disease activity parameters were not associated with changes in atherogenic index.

Table 2. Disease activity parameters and lipid levels during three months treatment with etanercept

	Baseline (N=92)	1 month	3 months	Regression coefficient (95%CI)	P value
Disease activity markers and acute phase proteins					
CRP, median (IQR)	13.0 (3.0–35.0)	2.0 (1.0–4.0)	2.0 (1.0–7.0)	-3.3 (-4.3– -2.6)	<0.001
HsCRP, median (IQR)	11.3 (3.1–33.2)	1.4 (0.8–4.2)	1.6 (0.8–5.4)	-4.3 (-6.0– -3.1)	<0.001
ESR mean (SD)	23.5 (19.0)	8.3 (9.5)	9.4 (11.6)	-14.5 (-17.6– -11.4)	<0.001
SAA, median (IQR)	4.8 (1.6–17.8)	0.9 (0.4–2.5)	0.8 (0.2–2.2)	-5.4 (-8.0– -3.7)	<0.001
BASDAI, mean (SD)	6.0 (1.5)	3.9 (2.1)	2.8 (2.0)	-3.2 (-3.6– -2.8)	<0.001
Lipid levels					
TC (mmol/l), mean (SD)	4.87 (0.88)	5.07 (0.90)	5.10 (0.87)	0.26 (0.14– 0.39);	<0.001
HDL-c (mmol/l), mean (SD)	1.29 (0.42)	1.35 (0.44)	1.42 (0.47)	0.10 (0.047– 0.15);	<0.001
LDL-c (mmol/l), mean (SD)	2.92 (0.79)	2.90 (0.82)	2.93 (0.78)	0.11 (0.004–0.22)	0.04
Triglycerides (mmol/l), median (IQR)	1.17 (0.89–1.74)	1.28 (0.97–2.15)	1.37 (0.84–2.07)	-0.90 (-0.99– -0.80);	0.03
apoA-1 (g/l), mean (SD)	1.39 (0.30)	1.46 (0.31)	1.48 (0.31)	0.077 (0.025–0.13);	0.004
apoB (g/l), mean (SD)	0.88 (0.21)	0.87 (0.21)	0.86 (0.20)	-0.002 (-0.026–0.022);	0.89
TC/HDL-c ratio, median (IQR)	3.89 (3.01–4.90)	3.85 (2.98–4.89)	3.71 (2.77–4.68)	-0.008 (-0.022–0.007);	0.32
apo-B/apoA-1 ratio, mean (SD)	0.67 (0.23)	0.63 (0.21)	0.62 (0.22)	-0.035 (-0.061– -0.009);	0.008

CRP, C reactive protein; HsCRP (high sensitivity CRP); ESR, erythrocyte sedimentation rate; SAA, serum amyloid A; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index, 0-10 scale; TC, total cholesterol; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; apoA-1, apolipoprotein A1; apoB, apolipoprotein B; TC/HDL ratio, ratio of TC/HDL; CI=confidence interval.

Values are mean (SD) or median (interquartile range (IQR)), as applicable. Regression coefficients were calculated using GEE analysis. Timepoint 0 months was used as reference category. Given p values account for 3 month values compared to the reference.

Table 3. Influence of disease activity parameters on lipid levels

	Lipids	Absolute change (mmol/l)	Relative change (%)	P value
Decrease in disease activity parameter				
-10 mg/l CRP	TC	0.10	+2.1	<0.001
	HDL-c	0.04	+3.1	<0.001
	apoA-1	0.04	+2.9	<0.001
	apoB/apoA-1 ratio	-0.02	-3.0	<0.001
-10 mm/hr ESR	TC	0.15	+3.1	<0.001
	HDL-c	0.05	+3.9	<0.001
	apoA-1	0.05	+3.6	<0.001
	apoB/apoA-1 ratio	-0.02	-3.0	<0.001
-10 mg/l SAA	TC	0.06	+1.2	0.003
	HDL-c	0.02	+1.6	0.01
	apoA-1	0.03	+2.2	<0.001
	apoB/apoA-1 ratio	-0.01	-1.5	<0.001
-1 point BASDAI	TC	0.06	+1.3	<0.001
	HDL-c	0.03	+1.9	0.001
	apoA-1	0.02	+1.3	0.001
	apoB/apoA-1 ratio	-0.008	-1.2	0.007

Depicted is the effect of a certain decrease in the separate disease activity markers on lipid levels (depicted as absolute and relative changes (with baseline values as reference)) as calculated using GEE analysis. CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; SAA, serum amyloid A; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index, 0-10 scale; TC, total cholesterol; HDL-c, high-density lipoprotein cholesterol; ApoA-1, apolipoprotein A1; ApoB, apolipoprotein B.

Surface-enhanced laser desorption/ionization time-of-flight analysis

Additional analyses were performed in a subgroup of 10 patients including 5 patients with high CRP levels at baseline (CRP > 30 mg/L) and 5 patients with low levels of CRP (<15 mg/L) at baseline. After SELDI-TOF analyses, protein spectra from HDL were obtained. Figure 1 shows the HDL profile and the respective plasma SAA levels of 3 representative patients over time. At baseline, a higher density of marker 11,695 m/z (which represents SAA) was found in the subgroup of AS patients with high CRP levels. During treatment all spectra showed virtually similar profiles and marker 11,695 m/z (SAA) disappeared from HDL as inflammation regressed in the patients with raised CRP at baseline (patients A and B in figure 1). Moreover, in patient B also a proteolytically-generated isoform of SAA appears to be present. It is known one-to-three aminoacids can be cleaved from either the N- or C-terminus of SAA (19).

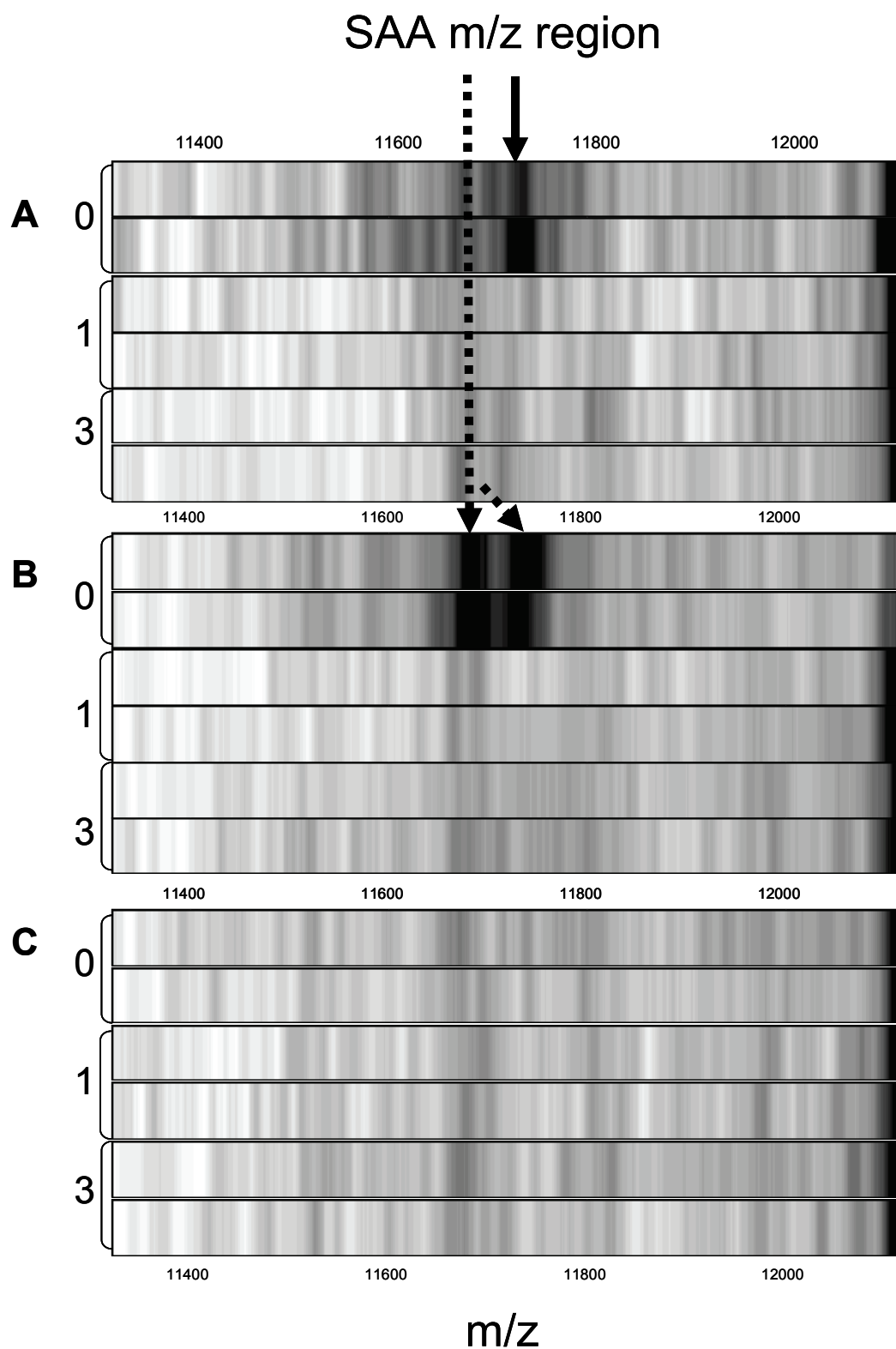


Fig. 1a. Representative examples of HDL spectra in gel view of three individual patients (A, B and C). Depicted are the spectra in the specific M/Z mass range of SAA (arrows). Each spectrum was measured in duplicate at time point 0 (baseline), 1 (one month after initiation of anti-TNF treatment) and 3 (three months after initiation of anti-TNF treatment). All spectra were normalized on total ion current.

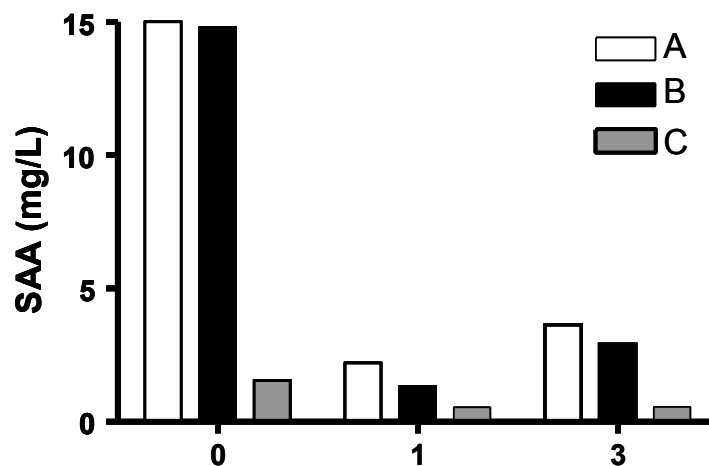


Fig. 1b. SAA levels. The overall plasma SAA levels over time (values on X-axis represent time point 0, 1 and 3 months after initiation of anti-TNF treatment) of the three representative AS patients (A, B and C).

DISCUSSION

The present study showed that AS patients with high (inflammatory) activity were characterized by decreased levels of TC, HDL-c and apoA-1 accompanied by biochemical changes of the HDL particle. Along with improvement of the lipid profile, reflected by increased HDL-c and apoA-1 levels and an improved apoB/apoA-1 ratio, anti-TNF treatment led to favourable alterations in HDL composition, i.e. diminishing of the SAA concentration within the HDL particles.

Our study is the first investigating alterations in apolipoprotein levels in AS patients, during anti-TNF treatment. ApoA-1 is the major atheroprotective apolipoprotein in the HDL particle, whereas apoB reflects the total number of potentially atherogenic particles as it is present in Very Low Density Lipoprotein (VLDL), Intermediate Density Lipoprotein (IDL) and Low Density Lipoprotein (LDL). Comparable to the TC/HDL-c ratio, the apoB/apoA-1 ratio has emerged as a very good predictor for future CV events, with the practical advantage that fasting blood samples are not required (20-23). This ratio reflects the balance of cholesterol transport in a simple way. The higher the apoB/apoA-1 ratio, the more cholesterol is circulating in the plasma compartment and this cholesterol is likely to be deposited in the arterial wall, causing atherogenesis and risk of CV events. In AS, apoB/apoA-1 ratio was positively associated with disease activity parameters and a 7.5% decrease of this ratio was accomplished during anti-TNF treatment, suggesting a beneficial effect on the risk for CV morbidity and mortality, although, due to the relatively small change in apoB/apoA-1 ratio, this should be interpreted cautiously.

Anti-TNF treatment resulted in a less atherogenic lipid profile, which is consistent with previous findings (10). Although the observed changes in lipid levels were small, even these small changes may well have a clinically relevant impact on the CV risk, since AS is a chronic inflammatory disease stretched out over many years (24). However, besides or even

beyond focusing solely on HDL-c levels, it seems important to investigate actual HDL composition and thereby its functional characteristics, to learn more about its effects on the vascular system and CV risk.

HDL protein profiling is increasingly used to determine the biochemical composition of HDL (18;25;26). During acute systemic inflammation HDL becomes pro-inflammatory, loses its protective properties and can even enhance atherogenesis (12;27). Interestingly, in addition to showing decreased plasma levels of HDL-c during active disease in AS, SELDI-TOF analysis enabled us to show actual alterations in HDL composition; i.e. in contrast to AS patients with low CRP levels at baseline, which exposed virtually no SAA on their HDL, SAA was markedly present on the surface of HDL in AS patients with increased CRP levels at baseline, but after inflammatory suppression this SAA almost disappeared in these patients. SAA is an acute phase reactant, synthesized mainly in the liver in response to proinflammatory cytokines such as IL-1, IL6 and TNF α (28) and elevated levels of SAA are associated with increased CV risk (29). SAA is transported mainly in HDL as an apolipoprotein (30;31). Increased serum SAA levels during the acute-phase response in patients with active AS thus seem to be accompanied by an increased presence of SAA within the HDL particle. Recently, an increased presence of SAA within the HDL particle was also found in patients with active Crohn's disease, another chronic inflammatory disease, which is associated with spondylarthropathies among which AS (32). This is interesting, since it is known that SAA is able to replace anti-atherogenic apoA-I in the HDL particle, which renders them less protective (33;34). Moreover SAA-rich HDL particles are rapidly cleared from plasma, and thus the increase in SAA during inflammation could also contribute to the decrease in total HDL-c concentrations (35). However, probably other mechanisms play a role in decreased HDL-c levels during inflammation as well. It has been suggested that remodeling of HDL through activation of secretory phospholipase A2 may be an alternate explanation for decreased HDL-c levels during the acute phase response. Overexpression of this enzyme in mice leads to decreased HDL-c levels and enhanced HDL-c catabolism (27;36-38). In addition, inflammation may convert HDL de novo into a more proatherogenic form by coordinate but inverse transcriptional regulation of SAA and apoA-1 in the liver (28). This may explain the observed inverse correlation between plasma levels of SAA and apoA-1, but not with HDL-c at baseline. Changes in TC, HDL-c and apoA-1 levels were significantly inversely associated with changes in disease activity parameters over time, confirming the role of inflammatory activity on changes in lipid profile.

In conclusion, the present study showed for the first time that during anti-TNF treatment for AS, along with favourable changes in lipid profile, HDL composition is actually altered, with SAA disappearing from the HDL particle, rendering it more atheroprotective. These findings

underline the importance of understanding the role of functional characteristics of HDL cholesterol in CV diseases, related to chronic inflammatory conditions such as AS.

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Chapter 1.4

ESR, CRP and SAA for patient selection and monitoring of anti-TNF treatment in Ankylosing Spondylitis

M.K. de Vries
I.C. van Eijk
I.E. van der Horst-Bruinsma
M. J. L. Peters
M.T. Nurmohamed
B. A.C. Dijkmans
B. P.C. Hazenberg
G.J. Wolbink

ABSTRACT

Objectives. To study usefulness of erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and serum amyloid A (SAA) for response prediction and monitoring of anti-TNF treatment in Ankylosing Spondylitis (AS) patients.

Methods. Patients were included consecutively before start with etanercept or infliximab treatment. ASAS response, defined as 50% improvement or an absolute improvement of 2 points of the BASDAI (0-10 scale), was assessed at 3 months. Inflammatory markers and BASDAI were collected at baseline, 1 and 3 months. Longitudinal data analysis was performed to compare associations between inflammatory markers and the BASDAI over time by calculating standardized betas. Predictive values of baseline levels of inflammatory markers for ASAS response were calculated.

Results. In total 155 patients were included of whom, after 3 months treatment 70% responded in the etanercept and 71% in the infliximab cohort. All markers, notably SAA, decreased significantly ($p < 0.0001$). Standardized betas were 0.49 for ESR, 0.43 for CRP and 0.39 for SAA. Normal baseline levels of CRP and SAA were significantly associated with non-response. A combination of elevated CRP and SAA levels at baseline revealed the highest predictive value (81%) for ASAS response.

Conclusions. ESR, CRP and SAA were significantly associated with BASDAI over 3 months, and association with ESR was the strongest. Elevated baseline CRP and SAA levels revealed the highest predictive value for response. Together, this study demonstrates that inflammatory markers, and notably CRP and SAA, may facilitate patient selection and monitoring of efficacy of anti-TNF treatment in AS and could be added to response criteria.

INTRODUCTION

Disease activity in ankylosing spondylitis (AS) is generally measured with the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) (1). Despite the fact that the BASDAI is a validated instrument used in many clinical trials as an outcome parameter for disease activity, it remains a subjective parameter that is based on a patient questionnaire. A previous study showed that the BASDAI has a high intraindividual week-to-week variability (2). Theoretically, a high BASDAI can be caused by three factors, including a high level of (a) ankylosis or joint destruction, or (b) psychological stress or, (c) inflammation. There is an unmet need for more objective biomarkers of disease activity in AS similar to the erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP) in rheumatoid arthritis. ESR and CRP are sensitive markers of disease activity in RA and are a reflection of the plasma levels of pro-inflammatory cytokines rendering them suitable for monitoring effectiveness of anti-tumour necrosis factor (TNF) drugs. In AS, however, sensitivity of these inflammatory markers as biomarkers of disease activity is controversial (3). On one hand, ESR and CRP are poorly associated with disease activity in AS and on the other hand, they may help a clinician to predict the response on TNF blockers (4-7). Two studies reported a strong association between ESR or CRP levels at baseline and clinical response to treatment with anti-TNF after 3 months supporting a potential distinctive exploitation of these biomarkers in identifying AS patients suitable for treatment with anti-TNF (8;9) which is of particular relevance, also in light of costs of biologicals and side-effects of these drugs. However as changes of CRP may be too small to be detected in AS with common methods, measurement of high sensitivity (hs) CRP might be a more appropriate marker for disease activity. Besides ESR and CRP, other inflammatory markers are known, such as serum amyloid A protein (SAA) (10). SAA is an acute phase reactant, which is mainly transported as an apolipoprotein in HDL and is synthesized predominantly in the liver by hepatocytes in response to proinflammatory cytokines (11). SAA was shown to correlate with disease activity in AS and one study even suggested superiority of SAA to ESR and CRP (4;12). However, longitudinal data and effects of treatment with anti-TNF agents on these relationships are lacking. Therefore, we explored the usefulness of ESR, CRP, hsCRP and SAA for monitoring of inflammation in AS patients treated with anti-TNF along with the association between these inflammatory markers and the BASDAI over time. In addition, the relation between elevated levels of these markers at baseline and Assessment of Ankylosing Spondylitis (ASAS) response was studied in these patients after 3 months of treatment with etanercept or infliximab in order to predict efficacy of this treatment.

PATIENTS AND METHODS

Patients and study protocol

Consecutive AS patients attending the outpatients' clinics of the Jan van Breemen Institute and VU University medical centre scheduled for treatment with etanercept were included and followed prospectively, as well as AS patients scheduled for treatment with infliximab in the VU University medical centre. All patients fulfilled the 1984 modified New York criteria (13) and started anti-TNF therapy according to the ASAS consensus statement on the initiation of TNF blocking agents in AS (14). Patients were treated with 25 mg etanercept twice a week, 50 mg etanercept once a week or infliximab 5mg per kilogram body weight every 6 weeks after a starting regime. None of the patients was treated with adalimumab, because adalimumab was not reimbursed for yet at the start of this study.

The study was approved by the medical ethical committee of both participating centers and all patients gave written informed consent.

Outcome measures

The primary outcome measure was clinical response after 3 months of treatment with etanercept or infliximab, according to "International ASAS Consensus Statement for the use of TNF-agents in patients with AS" which is equivalent to the Dutch guidelines for continuation of TNF blocking agents. In this consensus statement, ASAS response was defined as a 50% improvement or as an absolute improvement of 2 points of the BASDAI (0-10 scale) and an expert opinion in favour of continuation of treatment after 3 months (15;16).

Data and sera were collected at baseline, after 1 and 3 months of treatment. During every visit questionnaires on disease activity (BASDAI) were obtained. ESR and CRP were determined routinely. HsCRP and SAA were measured in patient's sera at baseline, 1 and 3 months. Collected sera were frozen at -20°C until testing. Commercially available kits were used to measure these inflammatory markers.

Analysis of ESR, CRP, hsCRP and SAA

ESR was measured with the Westergren method. Values are expressed in millimetres/hour (mm/h). A value below 15 mm/hour was considered to be normal, according to the cut-off used at the Jan van Breemen Institute.

Serum CRP levels were determined using the Roche/Hitachi, Modular P (VUmc) or cobas c (JBI) analyzers, based on the principle of particle-enhanced immunological agglutination (Roche Diagnostics GmbH, D-68298, Mannheim). Values are expressed in milligrams/litre. A CRP level below 10 mg/L was considered to be normal, according to the cut-off used at the Jan van Breemen Institute.

HsCRP levels were determined using the Roche/Hitachi cobas c systems with a detection range of 0.15-20 mg/L. The test principle consists of a particle enhanced immunoturbidimetric assay. Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies.

SAA levels were assessed with an enzyme-linked immunosorbent assay (ELISA) as described previously (17). A value below 4 mg/L was considered to be normal.

Statistical analysis

Continuous variables were reported as the mean \pm SD or, if skewed, as the median (inter quartile range (IQR)). Categorical variables were calculated as frequencies and percentages. The distribution of variables was tested for normality and transformed if necessary and possible. As the distribution of all inflammatory markers was skewed, Wilcoxon Signed Rank test was performed to investigate paired samples. Relative changes (%) of inflammatory markers were calculated. Generalized estimating equations (GEE) were performed to investigate the longitudinal relation between the inflammatory markers and BASDAI over a period of 3 months by calculating standardized betas. We investigated the possible influence of demographic or clinical variables: i.e. gender, age, ethnicity, HLA-B27, presence of peripheral arthritis and disease duration.

Logistic regression analysis was performed to investigate the association between the baseline levels of the inflammatory markers and the dichotomous outcome variable of ASAS response. Odds Ratios (OR's) and 95% confidence intervals (95% CI's) were calculated for the association between elevated baseline levels of the inflammatory markers and ASAS response. For ESR, CRP and SAA as predictors of ASAS response, sensitivity, specificity were calculated and ROC curves were constructed. In addition, predictive values of normal or elevated levels of CRP and/or SAA were calculated for ASAS response. Statistical analyses were performed with SPSS 15.0 software. The threshold for significance was set at $p < 0.05$.

RESULTS

In total, 155 patients were included and monitored after the start with anti-TNF treatment. The demographic and clinical features are shown in Table 1. During treatment, all pharmacological treatment remained unchanged.

The etanercept cohort comprised 117 patients and the infliximab cohort 38 patients. After 3 months 70% (80/115, as in 2 patients ASAS response was missing) and 71% (27/38) of the patients achieved ASAS response in the etanercept and infliximab cohort, respectively.

Table 1. Demographic and clinical assessments of AS patients at baseline, 1 and 3 months of treatment with etanercept or infliximab.

Variables	Baseline N=155	1 Month	3 Months
Male gender (number, %)	101 (65)		
Age (yrs, mean \pm SD)	42 \pm 11		
Caucasian (number, %)	125 (81)		
HLA-B27 positive (number, %)	123 (85)		
Presence of arthritis (number, %)	88 (58)		
Disease duration (yrs)	8 (3-16)		
BASDAI	6.2 (5-7.1)	3.4 (1.8-5.7)*	2.8 (1.4-4.3)*
ESR	21 (7-38)	6 (2-12)*	5 (2-13)*
CRP	15 (5-38)	3 (1-5)*	4 (2-6)*
HsCRP	14.3 (3.8-39.2)	1.9 (0.8-4.8)*	1.9 (0.9-5.8)*
SAA	7.5 (1.9-26.0)	0.7 (0.2-1.8)*	0.8 (0.2-2.0)*

Unless otherwise indicated, the values are the median (inter quartile range). HLA-B27, Human Leukocyte Antigen B27; arthritis, peripheral arthritis; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index (0-10); ESR, Erythrocyte Sedimentation Rate, normal <15 mm/hour; CRP, C-reactive protein, normal <10 mg/L; High sensitivity CRP, normal <10 mg/L; SAA, serum amyloid A protein, normal <4mg/L. *p<0.0001 compared to baseline

Baseline levels of ESR, CRP and SAA were elevated in 113 (73%), 96 (62%) and 99 (64%) of the patients, respectively. In 29 (19%) patients none of these markers was elevated at baseline. All inflammatory markers and the BASDAI decreased significantly after start of anti-TNF therapy ($p<0.0001$, Table 1, Figure 1). Notably, the median relative decrease after 1 month was 36% for the BASDAI (Figure 1A), 67% for ESR (Figure 1B), 75% for CRP (Figure 1C) and 84% for hsCRP (Figure 1D), while SAA decreased with 90% (Figure 1E). Longitudinal linear regression analysis (GEE) showed significant association between BASDAI and ESR, CRP, hsCRP and SAA over time ($p<0.0001$). There were no confounders influencing this association. The standardized betas were 0.49, 0.43, 0.43 and 0.39 for ESR, CRP, hsCRP and SAA, respectively. Hereafter, only results of associations with CRP are presented as results for hsCRP did not differ from CRP.

In 2 patients treated with infliximab who showed a secondary increase of particularly CRP and SAA after initial normalization, antibodies against infliximab were detected (Figure 2A,B,C) (18).

Patients with an elevated baseline level of CRP (>10 mg/L) achieved ASAS response after 3 months of treatment significantly more often compared to patients with normal baseline CRP levels with an OR of 2.8 (95% CI: 1.3-5.7, adjusted for gender and age).

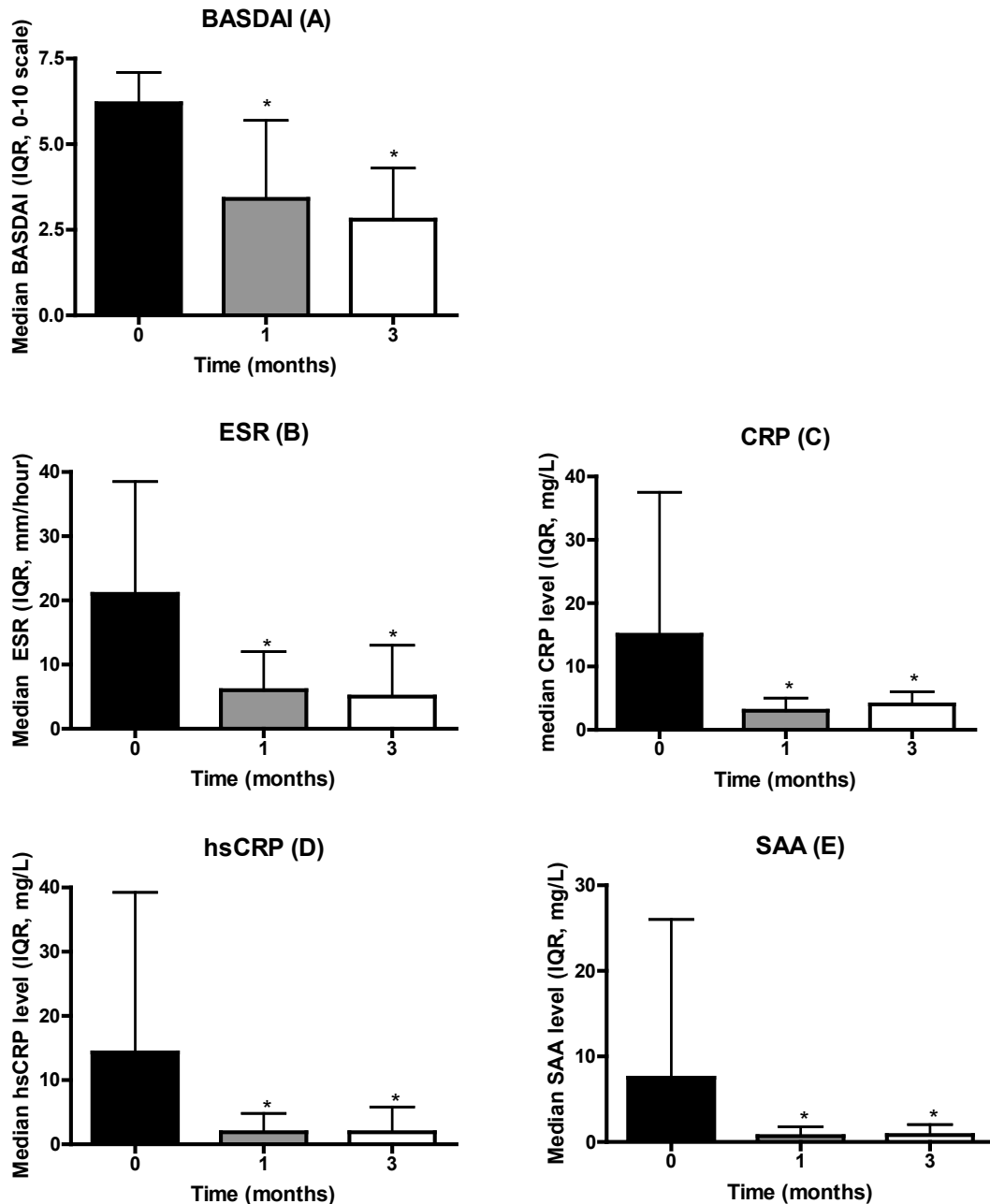


Figure 1. Change of BASDAI (A), ESR (B), CRP (C), hsCRP (D) and SAA (E) after 1 and after 3 months of treatment with etanercept or infliximab. (* $p < 0.0001$ compared to baseline).

Elevated baseline SAA levels had a similar association with ASAS response at three months of treatment with either infliximab or etanercept (OR 2.9 95% CI 1.4-6.1, adjusted for gender and age). Only baseline ESR levels were not significantly associated with clinical response (OR 1.4, 95% CI: 0.7-3.1). The sensitivity and specificity of CRP for prediction of ASAS response were 0.69 and 0.57 respectively. These figures were 0.72 and 0.54 for SAA, respectively.

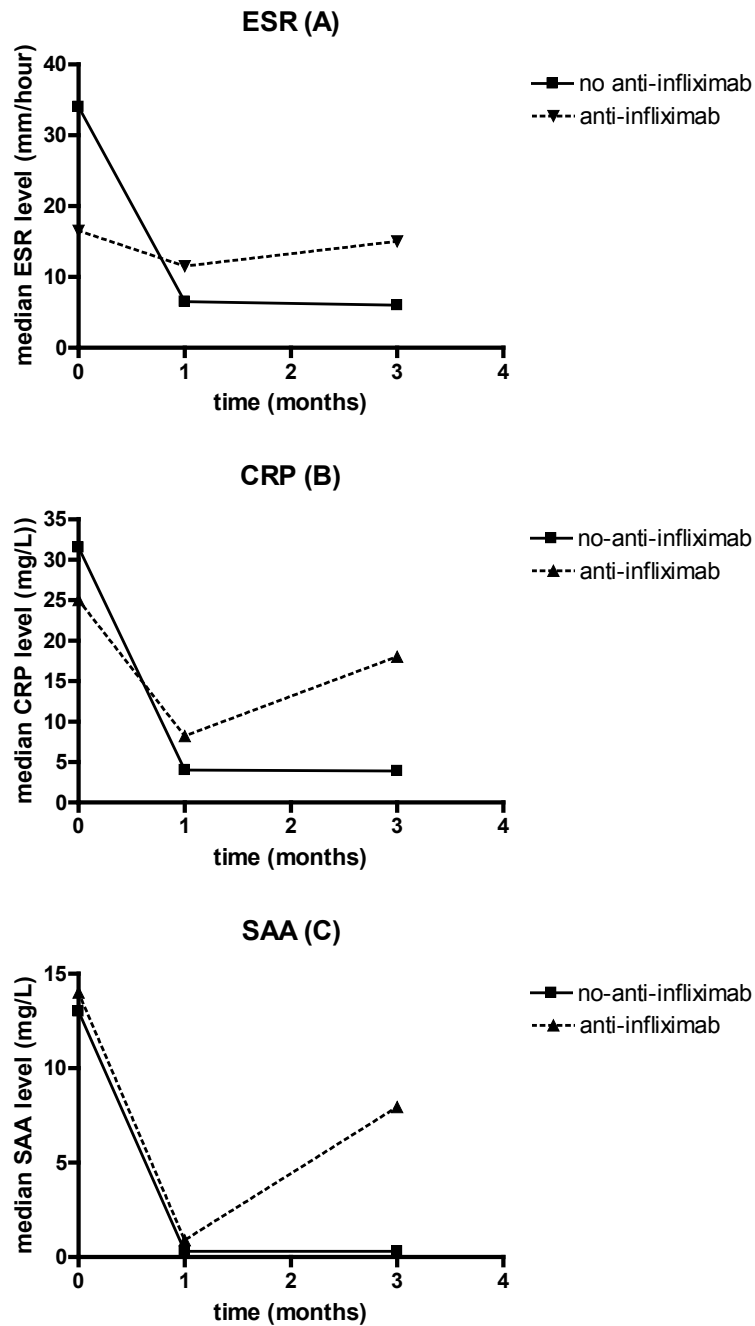


Figure 2. ESR (A), CRP (B) and SAA (C) levels 1 and 3 months after initiation of treatment with infliximab for AS patients without and with antibodies against infliximab (anti-infliximab, n=2).

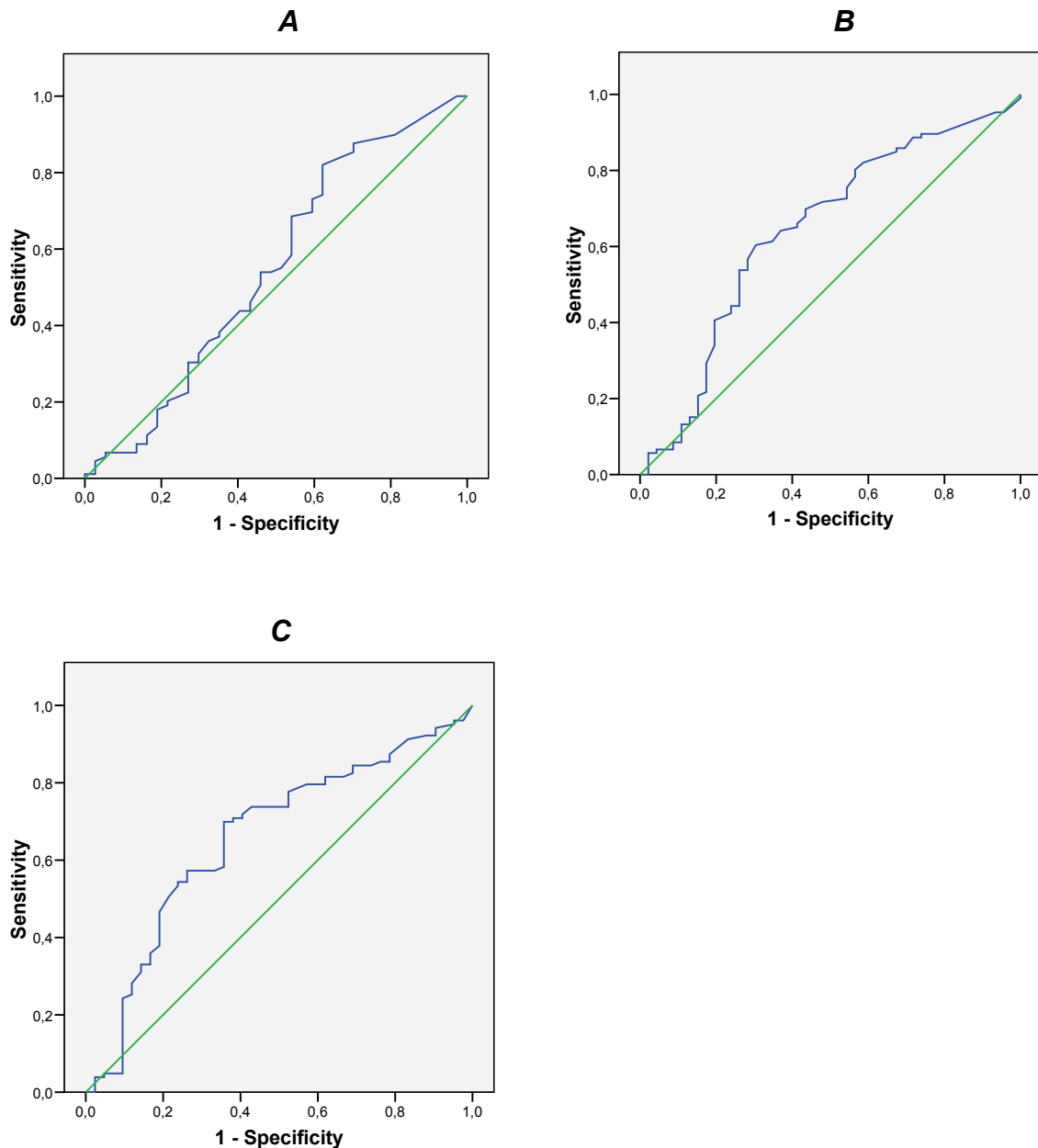


Figure 3. Receiver operating characteristic curves for A, erythrocyte sedimentation rate (area under the curve [AUC] 0.55), B, C-reactive protein (AUC 0.64), and C, serum amyloid A protein (AUC 0.66) as predictors of the ASessment in Ankylosing Spondylitisresponse.

ROC curves were created for ESR, CRP and SAA as predictors of ASAS response (Figure 3A,B,C). The ROC curve of ESR (Figure 3A) showed that determination of ESR is of no additional value in predicting ASAS response. CRP and SAA performed similarly (Figure 3B, 3C). The predictive value of ASAS response of an elevated baseline level of CRP was 79%. This value was identical to SAA. A combination of the presence of elevated baseline levels of CRP and SAA of patients in both cohorts displayed the highest predictive value of ASAS response of 81% (Table 2). Inclusion of baseline levels of ESR had no additional value. A

proportion of 48% of patients with normal values of both CRP and SAA at baseline were non-responders according to the ASAS criteria.

Table 2. Predictive values of normal or elevated pretreatment levels of CRP and/or SAA for prediction of ASAS response/non-response after 3 months of treatment with etanercept or infliximab.

	ASAS non-response	ASAS response
Normal CRP (N=59)	44%	56%
Elevated CRP (N=94)	21%	79%
Normal SAA (N=55)	45.5%	54.5%
Elevated SAA (N=98)	21%	79%
Normal CRP and SAA (N=44)	48%	52%
Elevated CRP, normal SAA (N=11)	36%	64%
Normal CRP, elevated SAA (N=15)	33%	67%
Elevated CRP and SAA (N=83)	19%	81%

ESR, Erythrocyte Sedimentation Rate, CRP, C-reactive protein, SAA, serum amyloid A protein

DISCUSSION

The present study demonstrated that a combination of elevated baseline levels of CRP and SAA can be a valuable instrument for selection of those AS patients who are likely to respond to treatment with anti-TNF, unlike the BASDAI. Moreover, inflammatory markers, CRP and SAA in particular, seem useful for monitoring the level of inflammation in patients with AS who are treated with etanercept or infliximab.

Monitoring

Regarding monitoring therapy with anti-TNF, most AS patients showed a significant decrease of several inflammatory markers, most dominantly SAA. In some cases, a secondary increase of these inflammatory markers can be seen, which might be caused by a concurrent infection or inadequate therapeutic levels. The latter may be due to antibody formation, which depletes anti-TNF levels below therapeutic thresholds (18). This could be an argument to include at least one inflammatory marker next to the BASDAI in order to assess disease activity properly. Though ESR showed the strongest association with BASDAI over time, we consider ESR the least suitable for inclusion as it has no additional value to the BASDAI, as the half-life of this inflammatory marker is too long for early detection of changes. HsCRP has been proposed to be useful as a marker to predict the risk of coronary heart disease due to inflammation in apparently healthy persons (19). In the current study however, measurement of hsCRP did not prove additional value as the strength of the association with disease activity of AS patients over time was not different from and not superior to that of CRP.

Although the majority of the AS patients in this study (62% up to 73%) had elevated inflammatory markers before start of anti-TNF therapy, it is known that inflammatory markers do not necessarily reflect disease activity well in AS (3). This is why inflammatory markers were not implemented for assessment of disease activity or response to treatment, which is in contrast to RA. This study shows that when inflammatory markers are raised this is indicative for active disease. It seems useful to add the decrease of inflammatory markers to response criteria for continuation of anti-TNF treatment in AS patients who show elevated inflammatory markers at baseline.

Patient selection

Since anti-TNF therapy is not without risks and is also very costly, it is of great importance to identify patients likely to (non)respond to this type of drugs. Although the performance of the tests was poor, the ROC curves showed that CRP and SAA are superior to ESR. In the present study we showed that the combination of elevated CRP and SAA levels at baseline is the strongest predictor of ASAS response, providing a solid basis for a predictive assessment of the clinical response of AS patients to treatment with anti-TNF. In contrast, baseline ESR levels were not associated with clinical response. However, as demonstrated before, patients with normal baseline levels of CRP and SAA may respond to anti-TNF therapy as well (20). Therefore, at this moment, we believe inflammatory markers can be very useful as one of the predictors of a good response, but a raise of the inflammatory markers should not be mandatory for allowing AS patients to be treated with anti-TNF.

The fact that good responders do not all necessarily need to have a strong decline of CRP or SAA, limits the use of these parameters in making the decision whether or not this therapy should continued. Therefore, they can be useful, but should not be considered obligatory for the decision whether anti-TNF therapy is failing or not.

SAA

We studied SAA in relation to disease activity in AS. SAA is implicated in several chronic inflammatory diseases, such as AA amyloidosis, atherosclerosis and rheumatoid arthritis (21). An additional advantage of monitoring SAA levels in AS patients may therefore be that SAA lowering therapy by anti-TNF could possibly prevent secondary AA amyloidosis. AA amyloidosis sometimes develops secondary to longstanding inflammation and chronically elevated levels of SAA, the plasma precursor of amyloid A deposits (10). Notably, elevated baseline levels of SAA were associated with clinical response in AS and decreased rapidly after initiation of treatment with etanercept or infliximab, which might prevent AA amyloidosis in the future.

Altogether, in this large prospective cohort of AS patients, measurement of inflammatory markers, in particular CRP and SAA, served as a powerful tool, not only for monitoring the efficacy of anti-TNF therapy, but also for selection of AS patients with a high likelihood of responding to anti-TNF treatment.

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SECTION 2

Cardiovascular risk in patients with rheumatoid arthritis

Chapter 2.1

**The metabolic syndrome is amplified in hypothyroid
rheumatoid arthritis patients: a cross sectional
study**

H.G. Raterman
I.C. van Eijk
A.E. Voskuyl
M.J.L. Peters
B.A.C Dijkmans
V.P. van Halm
S. Simsek
W.F. Lems
M.T. Nurmohamed

ABSTRACT

Objectives. Rheumatoid arthritis (RA) patients are at increased risk for cardiovascular disease (CVD), which is even more pronounced in hypothyroid RA patients. An unfavourable cardiovascular risk profile conferred by a higher prevalence of metabolic syndrome (MetS) and a higher Framingham risk score might explain this amplified cardiovascular morbidity. Hence, this study compared firstly, MetS (features) and, secondly, the Framingham 10-year CVD risk in RA patients with hypothyroidism compared to euthyroid RA patients.

Methods. RA patients participating in the CARRÉ investigation were divided in two groups: hypothyroid and euthyroid RA patients. MetS according to the NCEP ATIII-criteria and the Framingham risk score were compared between hypothyroid and non hypothyroid CVD event-free RA patients.

Results. In total, 257 RA patients were included: 236 RA (91.8%) and 21 hypothyroid RA (8.2%), respectively. The prevalence of MetS was significantly higher in hypothyroid RA patients (43%) compared to RA patients (20%). Moreover, female hypothyroid RA patients had a higher, Framingham risk score compared to euthyroid RA patients. With RA patients as reference category, the age and gender adjusted prevalence OR for MetS was 3.5 (95%-CI: 1.3 – 9.1) in hypothyroid RA.

Conclusions. Hypothyroid RA patients, particularly female patients, have a more unfavourable cardiovascular risk profile, reflected by increased prevalence of MetS and higher Framingham score, than euthyroid RA patients, suggesting a higher need for cardiovascular risk management in these patients to prevent future CVD events.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting approximately one percent of the general population. The manifestations of RA extend beyond a symmetrical inflammation of the joints as increasing evidence supports an increased risk for comorbid conditions like osteoporosis and cardiovascular diseases (CVD) (1-5). Hypothyroidism is another common comorbidity in RA and recent studies demonstrated that hypothyroidism is associated with CVD in RA (6;7). In euthyroid non-RA patients, associations between thyroid function and (components of) metabolic syndrome (MetS) (8), a constellation of several metabolic abnormalities, i.e. (central) obesity, hyperglycemia, hypertension and dyslipidemia (low HDL cholesterol and high triglycerides (TG) levels), were described. Although the existence of the MetS has been debated, a recent systematic review suggests that patients with MetS are at increased risk for a future cardiovascular event (9). Interestingly, there is data supporting an increased presence of MetS in inflammatory rheumatic diseases such as RA, systemic lupus erythematosus (SLE), and ankylosing spondylitis (AS) (10). Moreover, MetS (and its individual features) appeared to be associated with atherosclerosis in RA patients (6). A higher prevalence of the MetS in hypothyroid RA patients may therefore be a possible explanation for the increased cardiovascular morbidity in this subgroup. Hence, we examined the prevalence of the MetS (and its features) in RA patients with hypothyroidism relative to euthyroid RA patients and, in addition, we used the Framingham risk score, as an established tool for identifying high-risk individuals (11), to compare the estimated 10-yr CVD risk in these groups.

MATERIALS AND METHODS

Study population

Our study included RA patients (n = 353) participating in an ongoing prospective cohort study, i.e. the CARRÉ investigation, conducted at the Jan van Breemen Institute, investigating CVD and its risk factors in RA patients (2). The patients' ages ranged from 50 to 75 at the time of inclusion and RA was diagnosed according to the 1987 ACR criteria (12). At baseline patients' demographic, clinical and laboratory data (erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)) were collected. Disease activity was assessed by the 28 joints disease activity score (DAS28) (13). RA patients with a previous CVD event were excluded (n = 62), since these patients are on secondary prevention therapy interfering with (the features of) MetS and because the Framingham risk score is based on CVD event free patients. CVD was defined as a verified history of coronary, cerebral or peripheral arterial diseases. Coronary artery disease included a myocardial infarction, a coronary artery bypass

graft procedure or percutaneous transluminal coronary angioplasty. Cerebral arterial disease was defined as a cerebral vascular accident, a transient ischemic attack or carotid endarterectomy. Peripheral arterial disease included a peripheral arterial bypass, an ankle/brachial blood pressure index of less than 0.90 or leg amputation. As diabetes can be considered as a CVD event equivalent, and consequently are on secondary prevention therapy, patients with known diabetes ($n = 20$, ten of them already excluded for a CVD event) were excluded. Diabetes was defined as a known medical history of diabetes mellitus or the use of glucose lowering agent. To compare hypothyroid RA patients with euthyroid RA patients, (sub)clinical hyperthyroid RA patients ($n = 23$, two patients already excluded), defined as a decreased serum thyroid stimulating hormone ($TSH < 0.4$ mU/litre) or a documented medical history of clinical hyperthyroidism were excluded.⁷ Subjects of whom no blood samples were available ($n = 4$, one of them already excluded) or patients with both hypothyroidism and known DM ($n = 1$, already excluded for a CVD event) were also excluded, ultimately resulting in 257 RA patients eligible for this study. These patients were classified in two groups: RA (patients with RA alone) and hypothyroid RA (patients with both RA and hypothyroidism). Hypothyroidism was defined by a documented medical history of clinical hypothyroidism or by the presence of subclinical hypothyroidism. Subclinical hypothyroidism was defined by an increased serum TSH (> 4.0 mU/litre) in the presence of a normal ($11 - 25$ pmol/litre) serum free thyroxine (fT4) assessed from blood samples (7). This study was performed in accordance with the Declaration of Helsinki. All patients provided written informed consent and the Jan van Breemen Institute received approval by the local medical ethics committee.

Metabolic syndrome

The MetS was defined according to the original National Cholesterol Education Program – Third Adult Treatment Panel (NCEP ATP III) definition (14). According to this definition patients fulfil the criteria for MetS when three or more of the following factors are present:

- Abdominal obesity: in females waist circumference > 88 cm, and in males waist circumference > 102 cm;
- Raised blood pressure: systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg;
- Raised TG: ≥ 1.7 mmol/l (150 mg/dl);
- Reduced HDL cholesterol: in females < 1.29 mmol/l (50 mg/dl), and in males < 1.03 mmol/l (40 mg/dl);
- Raised fasting plasma glucose: ≥ 6.1 mmol/l (110 mg/dl).

The waist circumference was measured at the level midway between the lowest rib margin and the iliac crest. The mean value of 2 measurements was used. Double readings of the systolic and diastolic blood pressure were obtained on the right arm with the subject in sitting position after a 5 minute rest. For each patient fasting blood samples were collected for assessment of glucose, TG and HDL cholesterol levels. TG levels were measured by an enzymatic method using Roche clinical chemistry analysers and HDL cholesterol was determined enzymatically with PEG-modified enzymes.

Framingham risk score

The Framingham risk score is an algorithm, which includes age, gender, smoking, blood pressure and cholesterol, to estimate the 10-year cardiovascular risk in patients without prevalent CVD (11).

Statistical analyses

Characteristics of the two groups (RA and hypothyroid RA) were expressed as mean (SD) or median (interquartile range) and were compared using a Student's T-test, Mann Whitney U test or Chi square tests, when appropriate. With RA patients as the reference category, logistic regression analyses were performed to calculate prevalence odds ratios regarding the MetS for hypothyroid RA patients. Five separate models were used: 1) univariable analysis, using the crude, uncorrected data; 2) multivariable analysis, adjusting for age and gender; 3) adjusting for age, gender and DAS28; 4) adjusting for age, gender and current use of prednisolone; 5) adjusting for age, gender and cardiovascular risk management (defined as lipid lowering, blood pressure lowering or glucose lowering agents (15). A p-value below 0.05 was considered statistically significant. For all analyses SPSS 15.0 for Windows (SPSS, Inc., Chicago, Illinois) was used.

RESULTS

Characteristics

The main characteristics of the study population are summarized in Table 1. In total, 257 RA patients were included: 236 RA without hypothyroidism (91.8%) and 21 hypothyroid RA (8.2%).

Table.1. Clinical and demographic characteristics of RA patients

	RA N = 236	Hypothyroid RA N = 21	P-value
Demographics			
Age, years	62.1 (\pm 7.4)	63.8 (\pm 7.2)	0.32
Female, %	66.5	81.0	0.18
RA related features			
DAS28	3.8 (\pm 1.4)	4.2 (\pm 4.2)	0.24
ESR, mm/hour	16.9 (9.0 – 31.0)	21.0 (13.5 – 28.0)	0.16
CRP, mg/L	6.0 (3.0 – 16.8)	7.0 (5.0 – 23.0)	0.16
Cardiovascular risk factors			
MetS [†] , %	19.9	42.9	0.024
MetS features, n	1.0 (0.0 – 2.0)	2.0 (0.5 – 3.0)	0.09
Raised glucose [†] , %	9.7	14.3	0.46
Fasting Glucose, mmol/l	5.1 (4.7 – 5.5)	5.2 (4.9 – 5.8)	0.30
High waist circumference [†] , %	42.8	57.1	0.20
Waist circumference, cm	92.0 (82.0 – 101.0)	90.0 (83.5 – 104.0)	0.15
High blood pressure [†] , %	39.0	52.4	0.43
Systolic blood pressure, mmHg	137.5 (130.0 – 150.0)	145.0 (132.5 – 160.0)	0.14
Diastolic blood pressure, mmHg	80.4 (\pm 7.8)	83.6 (\pm 12.4)	0.26
Use of antihypertensive medication, %	16.1	14.3	1.00
High TG [†] , %	25.0	28.6	0.72
TG, mmol/l	1.31 (0.96 – 1.74)	1.32 (0.95 – 1.85)	0.97
Low HDL cholesterol [†] , %	23.7	42.9	0.05
HDL cholesterol, mmol/l	1.44 (1.16 – 1.78)	1.38 (1.07 – 1.68)	0.38
Use of medication for dyslipidemia	5.1	4.8	1.00
Framingham 10-year CVD risk,			
all patients	11.0 (7.0 – 15.0)	13.0 (9.0 – 16.0)	0.33
men	14.0 (11.0 – 22.0)	11.5 (6.75 – 23.75)	0.47
women	9.0 (6.0 – 13.0)	13.0 (10.0 – 16.0)	0.039

Continuous variables are presented as means with standard deviations in case of a normal distribution or as medians with inter quartile ranges (IQR) in case of a non-normal distribution. N = number; HDL = High Density Lipoprotein, % = percentage, [†] = according to the NCEP ATP III definitions

Hypothyroid RA versus RA

MetS was present in 42.9% of hypothyroid RA patients and in 19.9% of the RA patients ($p = 0.024$). Moreover, low HDL cholesterol was more prevalent in hypothyroid RA patients when compared to RA patients ($p = 0.053$). These results were similar after using the 2005 revised NCEP criteria (data not shown). Hypothyroid RA patients had a higher estimated median 10-year CVD risk, than non hypothyroid patients although this difference did not reach statistical significance. Subgroup analysis showed a significantly higher estimated 10-year CVD risk in female hypothyroid RA patients (13%) when compared to euthyroid female RA patients (9%) ($p = 0.039$).

Prevalence odds ratios for MetS in hypothyroid RA relative to RA

Hypothyroid RA patients had a more than threefold increased age and gender adjusted chance of having MetS as compared to RA patients (See Table 2, model 2). Adjustment for inflammatory markers, by means of DAS28, (See Table 2, model 3) and separately CRP and ESR (data not shown) did not influence these results. Further adjustment for glucocorticoids and drugs for cardiovascular risk management only slightly decreased the chance of having metabolic syndrome (See table 2 model 4 and 5). Replacement of glucocorticoids by the current use of DMARDs did not alter the results (data not shown).

Table 2. Prevalence OR's for MetS in hypothyroid relative to euthyroid RA patients

	OR	95% CI	p-value
Model 1			
RA	1.0		
Hypothyroid RA	3.5	1.4 – 9.2	0.009
Model 2			
RA	1.0		
Hypothyroid RA	3.5	1.3 – 9.1	0.011
Model 3			
RA	1.0		
Hypothyroid RA	3.5	1.3 – 9.2	0.011
Model 4			
RA	1.0		
Hypothyroid RA	3.1	1.2 – 8.3	0.018
Model 5			
RA	1.0		
Hypothyroid RA	3.1	1.2 – 7.8	0.020
Model 1 = crude data			
Model 2 = adjustment for age and gender			
Model 3 = like model 2 plus adjustment for disease activity (DAS28)			
Model 4 = like model 2 plus adjustment for current use of prednisolone			
Model 5 = like model 2 plus adjustment for cardiovascular risk management			
OR = Odds Ratio, CI = confidence interval			

DISCUSSION

This study demonstrated a substantially elevated prevalence of MetS in hypothyroid RA patients (43%) compared to patients with RA alone (19%). This increased prevalence of MetS in hypothyroid RA patients is an important finding, since the presence of the MetS increases the risk for a future CVD event. This is illustrated by the observations of a recent systematic review reporting that middle-aged individuals with MetS have a ~1.5 fold increased risk for cardiovascular events (9). In agreement, a Dutch community based study

with a similar study design as the present study, demonstrated that MetS was associated with a twofold higher 10-year risk of incident CVD (16). It is important to realize that, although MetS predicts future CVD risk, literature suggests that MetS does not enhance the predictive power for CVD of established scoring algorithms as the Framingham score. However, the advantage of MetS is that it serves well as a simple clinical tool for identifying high-risk subjects predisposed to CVD (17;18).

No prevalence data are available of MetS in hypothyroid patients without RA. However, in non-RA euthyroid populations decreasing serum levels of thyroxine are associated with an increased risk of MetS and with higher TG, lower HDL cholesterol levels and abdominal obesity, independent of the presence of insulin resistance in non-RA patients (8;19). A remaining, intriguing, question is whether the presence of a concomitant disease as hypothyroidism in RA amplifies the risk of MetS synergistically or independently. Unfortunately, we were unable to address this question since control groups with only hypothyroidism were lacking, making testing for interaction impossible.

Pathophysiological mechanisms explaining the development of MetS have not been fully understood. It has been established that inflammation influences several CVD risk factors, some of which are incorporated in MetS (20). Indeed, an inverse correlation between inflammatory markers and HDL cholesterol levels was observed (data not shown). This was expected, as inflammation leads to a deteriorated lipid profile with low HDL cholesterol levels (21-23). However, adjusting for inflammatory activity did not alter the odds ratios for having MetS in the hypothyroid group, suggesting that additional mechanisms are involved in the higher prevalence of MetS in hypothyroid RA patients. Similar results were observed, when DAS28 was replaced by ESR or CRP in the third model (data not shown). These results, however, need to be interpreted carefully due to the cross-sectional study design. Microvascular dysfunction is another interesting possible mechanism underlying the development of MetS, as it has been proposed to play a causative role in the association between hypertension, insulin resistance and the metabolic syndrome (24). Several rheumatic diseases, i.e. RA and AS, have been associated with impaired microvascular function and, interestingly, microvascular dysfunction appears to be present in hypothyroid patients as well (25-27). This may explain, at least partly, the amplified prevalence of MetS in RA with a concomitant disease like hypothyroidism.

A key message of the present study is that in daily clinical practice, particularly in RA patients with hypothyroidism, the MetS requires more attention, since the MetS reflects an increased, but theoretically modifiable, cardiovascular risk. Clinicians should be aware of the increased need for cardiovascular risk management in specific subpopulations of RA patients, like

hypothyroid RA patients, since relatively simple lifestyle and/or pharmacological interventions may prevent future CVD in this group of patients with an already enhanced background risk of CVD.

Several limitations need to be taken into consideration. First, the predictive and scientific value of MetS have recently been debated. This debate is based on the assumption that MetS has no improved predictive value for CVD events than established algorithms like Framingham and SCORE. Moreover, critics of the MetS state that the CVD risk of MetS is not greater than its individual features (28). Others reject the MetS as a clinical entity as there is no consensus on a common underlying feature. Second, although the Framingham risk score is an established tool for identifying people at risk for a future CVD event, the use of this algorithm for an estimation of the 10-yr CVD risk in this study, can be perceived as limitation as the Framingham score has not been validated for comorbid populations like ours. Finally, due to the low number of cases in the hypothyroid group, our results need to be interpreted with caution as independent associations between MetS and hypothyroidism can not be demonstrated. However, our conclusions are strengthened by the fact that the OR's are practically unchanged after adjusting for several possible confounders.

In conclusion, this is the first study reporting that concomitant hypothyroidism in RA patients is associated with a very high prevalence of MetS. Moreover, female hypothyroid RA patients have a higher estimated 10-yr CVD risk. These results suggest the need for an intensified cardiovascular risk management in hypothyroid (female) RA patients.

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Chapter 2.2

Microvascular Function is Preserved in Newly Diagnosed Rheumatoid Arthritis and Low Systemic Inflammatory Activity

I.C. van Eijk
E.H. Serné
B.A.C. Dijkmans
Y.M. Smulders
M.T. Nurmohamed

ABSTRACT

Objectives. Rheumatoid arthritis (RA) is associated with increased cardiovascular morbidity and mortality. Microvascular function has been linked to several risk factors for cardiovascular disease and may be affected in RA. It is, however, presently unknown at what point in the disease course abnormalities in microvascular function occur. We determined whether microvascular function is already disturbed in early DMARD-naïve RA-patients with low systemic inflammation.

Methods. Fifteen consecutive RA patients with a median symptom duration of 5 months, a C-reactive protein level of ≤ 20 mg/l and without a history of cardiovascular disease, and 15 age and sex matched healthy controls were recruited. Endothelium-dependent and endothelium-independent vasodilatation in skin was evaluated with laser Doppler fluxmetry after iontophoresis of acetylcholine and sodium nitroprusside, respectively. Videomicroscopy was used to measure recruitment of skin capillaries after arterial occlusion.

Results. CRP and ESR levels were mildly, but significantly elevated in patients compared to controls. No differences in both endothelium-dependent vasodilatation and capillary recruitment were observed between groups (709% vs 797%, $P=0.59$ and 37% vs 41%, $P=0.56$, respectively).

Conclusions. Skin microvascular function is preserved in early, DMARD-naïve RA patients with moderately active RA but low systemic inflammatory activity. Both the extent of the systemic inflammation and disease duration, therefore, may be important determinants of microvascular dysfunction and subsequent increased risk for cardiovascular disease.

INTRODUCTION

Cardiovascular disease (CVD) has been recognized as the major cause of excess morbidity and mortality in patients with rheumatoid arthritis (RA) (1-3). This increased cardiovascular risk in RA may partly be due to traditional CV risk factors, i.e. an atherogenic lipid profile and hypertension (4), but chronic inflammation is also thought to be important (5-9).

Inflammation-induced vascular dysfunction of the macrocirculation and microcirculation, in particular impaired endothelium-dependent vasodilatation, may predispose RA patients to CVD (4;10;11). Macrovascular endothelial dysfunction precedes and initiates atherosclerosis and is a predictor of long-term cardiovascular risk (12). Microvascular endothelial dysfunction, on the other hand, is important not only in the development of target-organ damage in the heart and kidney, but also in the development of cardiovascular risk factors such as hypertension and insulin resistance (13-15). Progressive impairment of microvascular endothelium-dependent vasodilatation of the skin is associated with an increasing coronary heart disease risk (16).

In longstanding RA, microvascular endothelium-dependent vasodilatation is impaired and associated with increased C-reactive protein (CRP) levels (17-19). Moreover, anti-inflammatory therapy improves both peripheral (cutaneous) and myocardial microvascular dysfunction in RA patients (20;21). This underlines the fact that the atherogenic process is not limited to plaque formation in large conduit vessels but is associated also with impaired microvascular function that plays an important role in regulating tissue perfusion, including myocardial perfusion and associated ischemia.

An intriguing question is at what point in the inflammatory disease course abnormalities in (micro)vascular function occur. Impaired microvascular endothelium-dependent vasodilatation can be demonstrated in patients with longstanding RA with low disease activity (20). In addition, a flare of inflammatory activity in RA patients impairs microvascular endothelium-dependent vasodilatation even more, whereas inflammatory suppression does not restore vasoreactivity completely to normal (18). In newly diagnosed RA patients and high systemic inflammation, both endothelium-dependent and endothelium-independent vasodilatation of the resistance vessels are impaired as assessed with venous occlusion plethysmography (10).

It is presently unknown whether microvascular dysfunction is already present in early DMARD-naïve RA-patients with moderate disease but low systemic inflammatory activity. The aim of the study was to establish whether microvascular endothelium-dependent vasodilatation and capillary recruitment is impaired in patients with very early, DMARD-naïve RA with low systemic inflammation compared to healthy controls.

PATIENTS AND METHODS

Subjects

Early untreated RA

Fifteen consecutive eligible patients (12 females) with RA were studied. Diagnosis was confirmed using the 1987 ACR criteria (22) and low systemic inflammatory activity was defined as a CRP level ≤ 20 mg/l. The vascular function study was performed < 2 weeks after the diagnosis of RA and prior to treatment with disease-modifying anti-rheumatic drugs (DMARDs) or oral corticosteroids. A total of 15 age- and sex-matched healthy subjects were studied as a control group. Clinical and biochemical characteristics of the study groups are shown in Table 1. Exclusion criteria were diabetes mellitus, hypertension, history of cardiovascular disease, Raynauds syndrome, scleroderma, concurrent infection, thyroid dysfunction and current or recent medication which might affect vascular function, except non-steroidal anti-inflammatory drugs.

All participants gave written informed consent and the study protocol was approved by the Medical Ethics Committee of the Slotervaart Hospital, Jan van Breemen Institute and BovenIJ Hospital.

Study Design

Microvascular measurements were conducted in a quiet, temperature-controlled room ($T=23.4\pm0.4^{\circ}\text{C}$) after 20-30 minutes of acclimatization, with the subjects in the sitting position and the investigated, non-dominant hand at heart level. Nailfold capillary studies and iontophoresis studies were performed on the same day by a single experienced investigator (IvE). Subjects were asked to refrain from beverages other than water (especially no caffeine or alcohol), smoking, medication, except acetaminophen if necessary and meals from midnight at the testing day. Nailfold capillaries in the dorsal skin of the third finger were visualized by a capillary microscope as previously described (23;24). Nailfold capillaries were recorded on videotape before and after 4 minutes of arterial occlusion with a digital cuff. This procedure was performed twice, and the mean of both measurements was used for analyses. In addition, venous congestion, with the digital cuff inflated to 60 mmHg for 60 seconds, was applied to expose a maximal number of non-perfused capillaries. Capillaries were counted by a single observer using the naked eye from a freeze-framed reproduction of the videotape and from the running videotape, when it was uncertain whether a capillary was present or not. Baseline capillary density was defined as the number of continuously erythrocyte-perfused capillaries during a 15- second period. Intermittently perfused capillaries were also visible and are proposed to form an important functional reserve that can be recruited during post-occlusive hyperaemia. The maximum number of capillaries

visible directly after cuff release was counted for 30 seconds. Post-occlusive capillary recruitment was calculated by dividing the increase in density by the baseline density. The day-to-day coefficient of variation (CV) of the capillary density in resting state was $2.3 \pm 1.8\%$. The CV of the percentage capillary recruitment and absolute capillary recruitment during post-occlusive hyperaemia were $8.3 \pm 4.9\%$ and $6.2 \pm 4.3\%$, respectively.

Endothelium-(in)dependent vasodilatation of forearm skin microcirculation was evaluated by iontophoresis of acetylcholine and sodium nitroprusside in combination with laser Doppler fluxmetry as previously described in more detail (25;26). Acetylcholine (1%, Miochol-E, Théa Pharma, Zoetermeer, the Netherlands) was delivered with an anodal current; 7 doses (0.1 mA for 20 seconds) were delivered, with a 60-second interval between each dose. Sodium nitroprusside (0.01%, Haagse ziekenhuis apotheek) was delivered with a cathodal current; 9 doses (0.2 mA for 20 seconds) were delivered, with a 90-second interval between each dose. Acetylcholine-dependent laser Doppler flux was measured on the non-dominant forearm, whereas nitroprusside-dependent laser Doppler flux was measured at the same spot on the opposite forearm, with approximately 15 minutes elapsed between the two measurements. The day-to-day CV of the percentage increase from baseline to the final two minutes of the plateau phase was $9.8 \pm 5.6\%$ for acetylcholine and $8.3 \pm 5.4\%$ for sodium nitroprusside.

Assessment of inflammatory parameters

Disease activity was measured by calculating the disease activity score of 28 joints (DAS28) (27).

CRP levels were determined using the Roche/Hitachi cobas 6000 analyzer, based on the principle of particle-enhanced immunological agglutination (Roche Diagnostics GmbH, D-68298, Mannheim, Germany). Values are expressed in milligrams/litre. A CRP below 10 mg/L was considered to be normal.

ESR was determined with local measurement techniques (Westergren method) and expressed in mm per hour.

Statistical Analyses

Data are expressed as mean (SD) or median (range) as appropriate. The distribution of variables was tested for normality and transformed if necessary. Student's t-test was used to compare continuous normally distributed variables within patients and matched controls. We used non-parametric Mann-Whitney U tests when appropriate. For dichotomous variables Pearson chi-square test was used. Correlations between variables were analysed by using Pearson correlation or Spearman's rho tests when appropriate. A two-tailed probability value of $P < 0.05$ was considered (statistically) significant.

RESULTS

Characteristics

Baseline, demographic and clinical characteristics of patients are shown in table 1. The mean DAS28 score was 4.7 and CRP and ESR levels were significantly higher in patients compared to controls. Median symptom duration was 5 months.

Table 1. Baseline characteristics of the RA patients and controls

Characteristics	Patients with arthritis (n=15)	Healthy controls (n=15)	P value
Age, years	48 ± 10	48 ± 10	0.92
Female, n (%)	12 (80)	12 (80)	N/A
Symptom duration, months	5 (4 -12)	N/A	N/A
Rheumatoid factor (RF) positive, n (%)	11 (73)	N/A	N/A
ACPA positive, n (%)	13 (87)	N/A	N/A
Erosive, n (%)	3 (20)	N/A	N/A
Disease activity score (DAS28)	4.7 (0.95)	N/A	N/A
ESR, mm/hour	18 (11 – 35)	5 (3 – 8)	<0.001
CRP, mg/l	3 (2- 17)	1 (1 – 2)	0.002
Fasting glucose	4.8 (4.2 – 5.3)	4.5 (4.2 – 4.7)	0.38
Total cholesterol	4.7 (4.0 – 5.3)	5.4 (4.1 – 5.7)	0.42
HDL-c	1.52 (1.31 – 1.87)	1.42 (1.27 – 1.88)	0.78
LDL-c	2.70 (2.11 – 3.37)	2.72 (2.01 – 3.88)	0.68
Triglycerides	1.01 (0.78 – 1.78)	0.99 (0.58 – 1.31)	0.26
Systolic blood pressure, mmHg	126 ± 15	118 ± 7	0.1
Diastolic blood pressure, mmHg	80 ± 8	79 ± 6	1.0
Smoking (%)	2 (13)	1 (7)	0.5
NSAID use, n (%)	10 (67)	N/A	N/A
Body mass index (BMI)	24.5 (22.4 – 27.4)	24.4 (22.1 – 25.2)	0.42

Data are mean +/-SD or median (interquartile range); ACPA, Anti-citrullinated protein/peptide antibodies; ESR, erythrocyte sedimentation rate; CRP, C reactive protein; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; pulse pressure = systolic blood pressure – diastolic blood pressure; NSAID, non-steroidal anti-inflammatory drug.

Microvascular function is not disturbed in very early, untreated arthritis

Endothelial (in)dependent vasodilatation

Microvascular vasodilatation in response to acetylcholine (endothelium dependent) in RA patients was comparable to controls (709% versus 797%, respectively, $P=0.52$; table 2 and figure 1). The response to sodium nitroprusside (endothelium independent) also did not differ significantly between patients and controls (1292% versus 1094%, $P=0.39$).

Capillary density and recruitment

Baseline capillary density was similar in both groups, 49/mm² in patients versus 46/mm² in controls. Absolute and relative post- ischemic capillary recruitment did not differ between patients and controls (17 versus 18, respectively for absolute increase, $P=0.71$ and 37% versus 41%, respectively for relative increase, $P=0.19$). The total number of anatomically present capillaries, visible after venous occlusion was 72 in patients versus 68 in controls, which was not different ($p=0.56$; table 2, figure 2).

No correlations between microvascular function and disease activity markers and symptom duration

We did not find significant correlations between CRP or ESR or DAS28 and endothelium-dependent vasodilatation or capillary recruitment. Endothelium dependent vasodilatation and capillary recruitment did not correlate significantly with symptom duration.

Table 2. Microvascular measurements

	RA patients	Healthy controls	P value
Ach-mediated vasodilatation	n=15	n=15	
Skintemperature °C	30.3 ± 0.9	29.9 ± 0.7	0.12
Baseline skin perfusion, PU	8.5 (3.7 – 14.5)	6.8 (4.6 – 8.0)	0.58
Ach-mediated vasodilatation, %	709 ± 454	797 ± 435	0.59
SNP-mediated vasodilatation			
Skintemperature °C	30.0 ± 0.9	29.8 ± 0.8	0.47
Baseline skin perfusion, PU	5.3 (3.6 -7.3)	6.7 (5.1 – 9.6)	0.25
SNP-mediated vasodilatation, %	1292 ± 772	1094 ± 638	0.45
Capillary recruitment	n=14	n=15	
Skintemperature °C	30.2 ± 1.4	29.7 ± 1.3	0.24
Baseline capillary density, number/mm ²	49 ± 11	46 ± 12	0.57
Peak capillary density, number/mm ²	66 ± 15	64 ± 16	0.80
Venous occlusion, number/mm ²	72 ± 16	68 ± 18	0.56
Absolute increase, number/mm ²	17 ± 8	18 ± 8	0.79
Capillary recruitment, %	37 ± 18	41 ± 18	0.56

Data are mean +/-SD or median (interquartile range); Variables were tested using Student's T-test or Mann-Whitney U test. Ach = acetylcholine; SNP = sodium nitroprusside. Peak capillary density was defined as the maximum number of capillaries visible directly after arterial occlusion during post-ischemic hyperaemia. Venous occlusion represents the maximal number of non-perfused capillaries exposed after venous congestion.

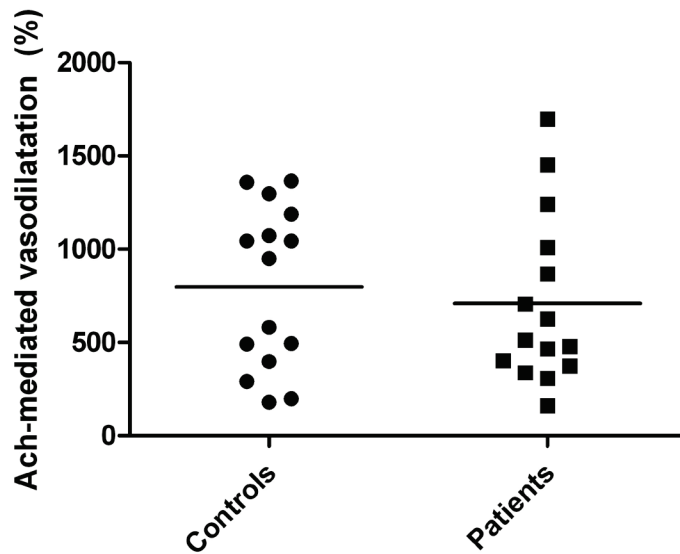


Figure 1. Acetylcholine-mediated vasodilatation in controls and RA patients. The mean of vasodilatation, indicated by the horizontal line, is comparable in patients and controls. ACh, acetylcholine.

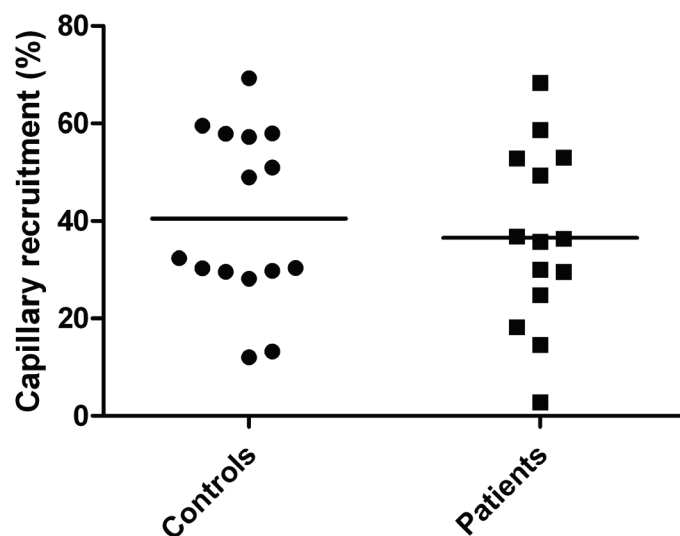


Figure 2. Capillary recruitment depicted as the percentual increase of visible capillary numbers between baseline and after post-occlusive hyperaemia.

DISCUSSION

In the present study we observed a preserved microvascular endothelium-dependent vasodilatation and capillary recruitment during reactive hyperemia in DMARD-naïve patients with newly diagnosed RA and low systemic inflammatory activity. Peripheral and coronary microvascular dysfunction is considered important in the development of cardiovascular disease (14;28). Previous studies demonstrated that coronary and peripheral microvascular dysfunction is apparent in longstanding established RA with high, but also low inflammatory

activity (11;18;19). Moreover, anti-inflammatory therapy improves microvascular function, but does not completely restore it (18;24). This persisting microvascular dysfunction may be caused by cumulative effects of inflammation, which in the long run lead to irreversible vascular damage. The finding of an inverse association between coronary microvascular function and disease duration in RA is compatible with such an explanation (20). On the other hand, microvascular dysfunction may already be present at the time of diagnosis, because subtle increased levels, sometimes within the normal range, of C-reactive protein can already be detected years before the onset of clinical RA (29). Our findings, however, do not support this, as we found preserved microvascular function in patients with newly diagnosed, moderately severe RA with low systemic inflammatory activity. Interestingly, CRP-levels in our newly diagnosed RA patients are comparable to the CRP-levels (4,2 mg/L) of patients with longstanding RA demonstrating impaired coronary microvascular function (20), suggesting that disease duration is an independent determinant of microvascular function. Nevertheless, newly diagnosed patients with RA do exhibit impaired endothelium-dependent and endothelium-independent vasodilatation of small arteries and resistance vessels if systemic inflammatory activity is more pronounced (i.e. CRP 29 ± 10 mg/L) (10). Moreover, suppression of inflammatory activity seems to restore vasodilatory function (10). These findings, together with our finding, suggest that systemic inflammatory activity is necessary to cause microvascular dysfunction, which is reversible early in the disease course, but becomes irreversible after a longer disease duration.

Obviously, with relatively low patient numbers a type 2 error may occur more easily. Although we cannot completely exclude a difference in endothelium-dependent vasodilatation between RA-patients and control subjects, it should be realised that the observed difference was very small (88 percentage points difference). To put this difference in perspective: in patients with longstanding RA, hypertension, obesity, or individuals at increased coronary heart disease risk the difference in endothelium-dependent vasodilatation, as compared with healthy controls, exceeds 300 percentage points (16;18;26;30).

In conclusion, the present study showed that skin microvascular function is not impaired in very early, DMARD-naive RA patients with low systemic inflammation, compared to healthy controls.

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Chapter 2.3

Case report:

Decrease of fructosamine levels during treatment with adalimumab in patients with both Diabetes and Rheumatoid Arthritis

I.C. van Eijk
M.J.L. Peters
M.T. Nurmohamed
A.W. van Deutekom
B.A.C. Dijkmans
R.J. Heine
S. Simsek

ABSTRACT

Tumour necrosis factor alpha (TNF α) is a pro-inflammatory cytokine which has been closely linked to obesity and insulin resistance. We present two cases of patients with rheumatoid arthritis (RA) and concomitant diabetes mellitus, who showed a marked decrease of fructosamine levels after initiating therapy with adalimumab, a TNF α -blocking agent, for active RA. This finding may implicate that TNF α –blockade causes better glycaemic control in RA patients with concomitant diabetes, possibly by improving insulin resistance.

INTRODUCTION

Tumour necrosis factor alpha (TNF α), a proinflammatory cytokine, plays an important role in inflammatory and auto-immune diseases like rheumatoid arthritis (RA). TNF α has also been closely linked to obesity and insulin resistance [1]. Increased insulin resistance, just as RA [2], is an important risk factor for developing cardiovascular disease (CVD) [3]. Thus far, the role of TNF α in insulin resistance has remained controversial. Despite a clear reversal of insulin resistance by TNF α neutralization in animal models [4,5], two studies in humans did not show an effect of administration of either a chimeric anti-TNF α antibody [6] or a recombinant soluble TNF α -receptor [7] on insulin sensitivity in obese or type 2 diabetes patients. However, recently it was shown that TNF α -infusion impairs glucose uptake in human skeletal muscle by altering insulin signal transduction [8] and induces insulin resistance in healthy volunteers [9]. Moreover, TNF α -antagonists, in addition to their known powerful anti-inflammatory effects, may have a beneficial effect on insulin resistance in rheumatic diseases. [10-12]. Beneficial clinical effects of treatment of RA with TNF α -antagonists on concomitant diabetes have not been described. We describe herein two cases of RA patients with concomitant diabetes in which glycaemic control parameters changed after initiation of adalimumab therapy.

RESEARCH DESIGN AND METHODS

A patient with known diabetes type 1 and concomitant RA showed a marked improvement of HbA1c levels after initiation of adalimumab, a recombinant human IgG1-monoclonal antibody, therapy for active RA when she visited the endocrinologist (SS) in the VUmc. Due to this finding, the effect of adalimumab on fructosamine levels was assessed through measuring fasting fructosamine levels over time in RA patients, according to the 1987 ACR-criteria, with concomitant diabetes before and during adalimumab therapy. A survey into a cohort of RA patients treated with adalimumab in the Jan van Breemen Institute, a large outpatient clinic for rheumatology, was conducted to identify RA patients with concomitant

diabetes, i.e. type 1 diabetes and type 2 diabetes according to WHO-criteria. Further inclusion criteria were (a), no concomitant use of prednisone around the start and during the use of adalimumab, (b) ≥ 3 stored, deep-frozen serial serum samples available, of which at least one < 1 month before and one during adalimumab therapy, and (c) use of a stable adalimumab dose in the period of serum sample collection. On further analysis, 5 of 7 patients initially recruited did not meet these criteria and were excluded: 3 patients used concomitant prednisone in decreasing dosage, 1 patient was noncompliant to adalimumab therapy and of 1 patient no serum sample was available < 1 month before start with adalimumab. Of the remaining 2 patients, fructosamine levels were determined in all available serum samples. Medical records were used to collect clinical data. Information regarding dietary habits and physical activity patterns was collected by phone.

Fructosamine measurement

As only serum was available fructosamine was chosen as reference for glycaemic control. The serum fructosamine concentration is an indicator of glycosylated serum protein and reflects the average blood glucose level over 1 to 3 weeks. Therefore, serum fructosamine level is a useful marker to assess short-term glucose control. Serum fructosamine levels were measured in VUmc by means of a validated, commercially available method.

Statistical analysis

To investigate whether fructosamine levels changed significantly before and after adalimumab treatment the following technique was used. First, the data of both patients was pooled. Subsequently, a regression line was drawn through all fructosamine values collected after the start with adalimumab (same time points for both patients). This regression line was extended towards the time-point corresponding with the “pooled” fructosamine level before the start with adalimumab using the most negative regression coefficient possible i.e. the lower bound of the 95% confidence interval (95%CI) of the regression coefficient. At this point we calculated the upper bound of the 95%CI. This value was compared with the observed value.

RESULTS

Two RA patients with diabetes were included. One 40 year old female with type 1 diabetes since 18 years (index patient) and one 66 year old male with type 2 diabetes since 9 months. These patients showed a substantial decrease in fructosamine levels after start with adalimumab (figure 1). Blood glucose lowering therapy consisted of 50 units of human insulin per day in patient 1 and glimepiride 2 mg per day in patient 2 and remained stable during the treatment period. Dietary and physical habits did not change.

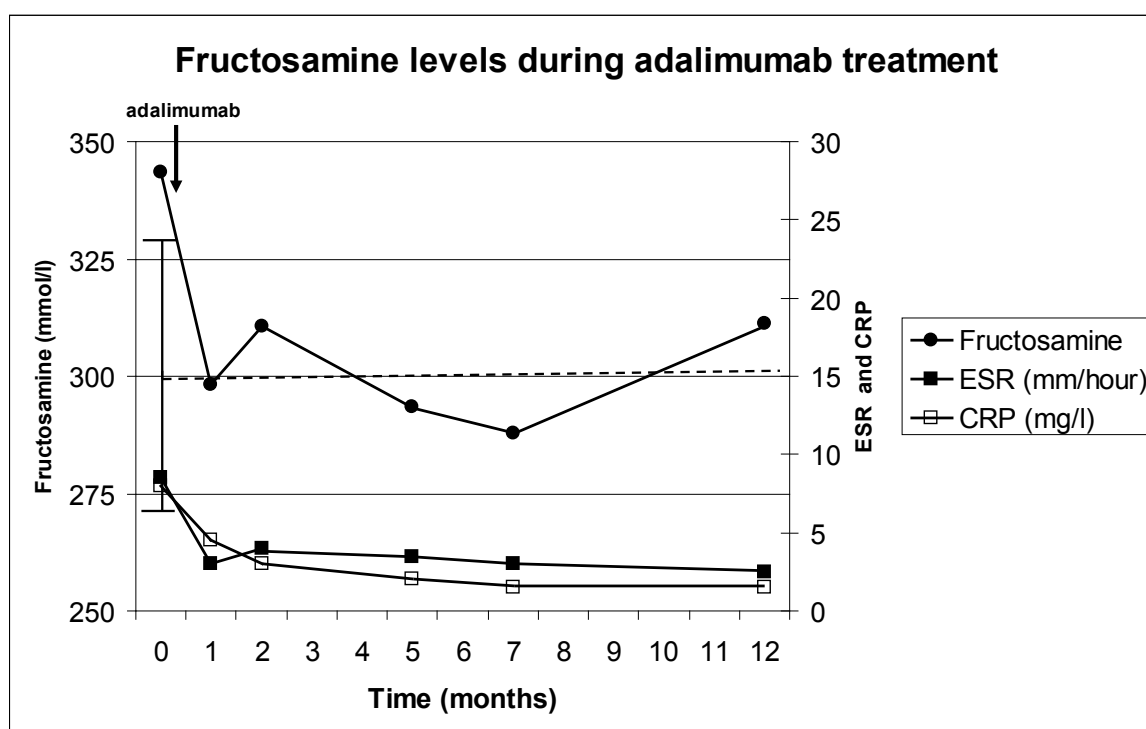


Figure 1. Pooled fructosamine, ESR and CRP levels in patient 1 and 2 over time (months). The arrow indicates the start with adalimumab.

CONCLUSION

Our results illustrate a fast and substantial decline in fructosamine levels in 2 patients with RA and concomitant diabetes after initiation of treatment with adalimumab. The improved glycaemic control is in accordance with a previous report of a psoriatic arthritis patient in which type 2 diabetes disappeared upon treatment with infliximab, suggesting an improvement of insulin sensitivity [12]. However, in this case report, the improvement of glycaemic control occurred after prolonged treatment (4-5 months) with infliximab, while we found a faster response. Type 2 diabetes is characterized by insulin resistance of the major target tissues and (relative) β -cell failure, leading to decreased insulin secretion. In general, TNF α plays a role in the pathophysiology of diabetes. TNF α synthesized and secreted by adipose tissue, regulates insulin receptor function by impairing insulin signalling [13]. Moreover, TNF α plasma levels are increased in type 1 diabetes and are associated with long-term glycaemic control parameters, among them HbA1c and fructosamine [14]. Furthermore it is clear that RA patients have enhanced serum levels of TNF α due to the presence of chronic systemic inflammation. Hence we hypothesize that TNF α -blockade has improved insulin signalling in both patients, subsequently leading to a decrease of fructosamine levels and thereby a better glycaemic control. This theory is supported by the

fact that 1) the administered glucose lowering therapy remained stable and dietary and physical activity patterns did not change around the start of adalimumab therapy and 2) inflammatory markers were not substantially elevated before treatment, implicating that their contribution to the decline of fructosamine levels probably is small. However, we cannot exclude the possibility that this improved glycaemic control is partly due to alterations in inflammatory activity. Our findings indicate that TNF α -blockade causes better glycaemic control in RA patients with concomitant diabetes by improving insulin resistance, which may explain why TNF α -antagonists decrease the risk of first incidence of CVD in RA [15]. Moreover, clinically, physicians should be aware of the possibility influencing diabetes by aggressive treatment of RA with TNF α -antagonists. Further prospective research is required.

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SECTION 3

Treatment strategy in
early inflammatory arthritis

Chapter 3.1

Aggressive therapy in patients with early arthritis results in similar outcome compared to conventional care: the STRategies in Early Arthritis Management (STREAM) randomized trial

I.C. van Eijk
M.M.J. Nielen
I.E. van der Horst-Bruinsma
G.J. Tijhuis
M. Boers
B.A.C. Dijkmans
D. van Schaardenburg

Submitted

ABSTRACT

Objectives. Aggressive treatment of rheumatoid arthritis (RA) prevents joint damage and functional decline, but its effect is uncertain in patients with early inflammatory arthritis, not necessarily meeting the ACR criteria for RA.

Methods. Patients with 2-5 swollen joints, Sharp-van der Heijde radiographic score (SHS) <5 and symptom duration < 2 years were randomized between 2 strategies. Patients with a definite non-RA diagnosis were excluded. The protocol of the tight control group aimed for remission (Disease Activity Score (DAS) <1.6), with consecutive treatment steps: methotrexate, addition of adalimumab, combination therapy. The conventional care group was treated with traditional DMARDs (no prednisone/biologics) without DAS-based guideline. Outcome measures after 2 years were SHS, remission rate and health assessment questionnaire (HAQ) score.

Results. 82 patients participated (60% ACPA positive). In the tight control group (n=42), 19 patients were treated with adalimumab. In the conventional care group (n=40), 24 patients started with hydroxychloroquine, 2 with sulfasalazine and 14 with methotrexate. After 2 years, median SHS increase was 0 (IQR 0-1.1) and 0,25 (IQR 0-2.8), remission rates were 66% and 49% and HAQ decreased with a mean of -0.09 and -0.125 in the tight control and conventional care group, respectively. All comparisons were statistically nonsignificant.

Conclusions. In patients with early arthritis of 2-5 joints, both tight-control therapy including adalimumab and conventional therapy resulted in remission rates around 50%, low radiographic damage, and excellent functional status after 2 years. However, full disease control including radiographic arrest in all patients remains an elusive target even in moderately active early arthritis.

INTRODUCTION

The early and aggressive treatment of patients with rheumatoid arthritis (RA) is increasingly successful, particularly with combinations of disease modifying antirheumatic drugs (DMARD), also containing anti-TNF therapy (1-5). Among the results are percentages of sustained remission of around 40%, excellent functional status and nearly complete arrest of radiological damage progression. In an attempt to explain these better results than had been attained before in RA of longer duration, the concept of a “window of opportunity” was proposed, suggesting that early suppression of active inflammation produces long-term benefits (6).

Intensive therapy, preferably with a combination of drugs, therefore is a well-established treatment strategy for patients with early active RA. However, the optimal strategy for patients presenting with only a few inflamed joints is not yet clear. This category of patients is more difficult to study due to the problem of classifying these patients as having RA or undifferentiated arthritis (UA) (7). In recognition of this issue, a combined task force of ACR and EULAR has developed new classification criteria for RA (8). One of the objectives of these criteria is to increase the sensitivity for the diagnosis RA in early UA, in order to facilitate the conduction of clinical trials in this category of patients. Other aspects to consider in trials in this group of patients are that around half of patients with UA will remit within one to two years (9-13), that it is inherently less possible to demonstrate a reduction of an already low disease activity, and finally that any toxicity of treatment is less acceptable since it is occurring in patients with only mildly active disease.

In a preceding study we have shown that patients with more severe forms of UA are undertreated in comparison to patients with RA (9). One clinical trial has shown that UA patients treated with methotrexate versus placebo have less progression to RA (according to 1987 ACR criteria (14)) and less progression of radiographic erosions, but these differences were confined to the subgroup of anti-citrullinated protein antibody (ACPA) positive patients (15). On the other hand, in early RA good results can also be obtained with the ‘milder’ drug hydroxychloroquine (16).

The present two-year trial investigated whether the approach of early aggressive therapy was also effective in arthritis patients presenting with only moderately active disease, i.e. in those patients who would not meet the usual inclusion criteria for trials in active RA.

PATIENTS AND METHODS

Patients

Eligible patients were 18 years or older, with a symptom duration of <3 years. In addition, they had to have 2 to 5 swollen joints and a total Sharp-van der Heijde radiographic score (SHS) (17) <5. Patients did not have to meet the 1987 ACR criteria for RA. Exclusion criteria were prior treatment with a DMARD, except for hydroxychloroquine, the use of corticosteroids in the last three months or an intra-articular injection with corticosteroids in the last month. In addition, patients with bacterial arthritis, crystal-induced arthritis, psoriatic arthritis, reactive arthritis, osteoarthritis, and arthritis due to sarcoidosis or another systemic autoimmune disease other than RA as well as pregnant patients and patients with a wish to conceive during the study were excluded. The patients were recruited from the rheumatology clinics of the Jan van Breemen Institute and the VU University Medical Center in Amsterdam, The Netherlands. The study was approved by the local institutional review board and all patients gave written informed consent. Trial registration number is NTR 144.

Study design and treatment algorithm

The study was designed in analogy to the “BeSt” study of treatment strategies in early active RA (3), and compared two treatment strategies in a single-blind clinical trial. Whereas in the BeSt study the criterion for a change of therapy was a disease activity score (DAS) of >2.4, here we used a lower DAS threshold for a change of therapy of 1.6, as disease activity is inherently lower in this group of patients. Also, the goal of the intervention was to achieve and maintain remission, which is defined as a DAS <1.6 (18).

The patients were randomised in blocks of 10 into one of two treatment groups: 1) aggressive therapy and 2) conventional care. In the aggressive group, therapy was aimed at achieving and maintaining a DAS (44 joint score) of <1.6, which is considered to represent remission (18). Every 3 months the DAS was performed by a research nurse who was blinded to the allocated treatment group. Treatment was started with oral methotrexate 15 mg/wk. If the DAS was ≥ 1.6 at a given time point, the therapy was changed (see also figure 1). The predefined steps were: increase of methotrexate to 25 mg/wk, methotrexate 25 mg/wk combined with adalimumab 40 mg/2 wk, methotrexate 25 mg/wk combined with adalimumab 40 mg/wk, a combination of methotrexate 25 mg/wk, sulfasalazine 2000 mg/day and hydroxychloroquine 400 mg/day, a combination of methotrexate 25 mg/wk, sulfasalazine 2000 mg/day, hydroxychloroquine 400 mg/day and prednisone 7.5 mg/day, leflunomide 20 mg/day and intramuscular gold 50 mg/wk, respectively. If the DAS was <1.6 at one time point the treatment remained unchanged. If the DAS was <1.6 at two consecutive time points the

following actions were taken, depending on the treatment step where the patient was at that moment: methotrexate 15 mg/wk was decreased with 2,5 mg/2wk to 0 mg/wk after 3 months, methotrexate 25 mg/wk was decreased with 2,5 mg/ 2 wk to 10 mg/wk after 3 months, adalimumab 40 mg/2 wk was stopped, adalimumab 40 mg/wk was decreased to adalimumab 40 mg/2wk, hydroxychloroquine was decreased with 200 mg/ 8 wk to 0, if remission was sustained after 3 months sulfasalazine was decreased subsequently with 500 mg/4 wk to 0, if remission was sustained after 3 months methotrexate was decreased with 2,5 mg/2wk to 0, prednisone 7,5 mg/day was decreased to 0 mg in 7 weeks, leflunomide was decreased to 10 mg/day, and if remission was sustained after 3 months leflunomide was stopped, gold was decreased to 50 mg/2wk, if DAS remained <1.6 gold was decreased to 50 mg/4wk, if remission was sustained gold was stopped. If at any time point the DAS was ≥ 1.6 the last effective treatment was restarted. In case of intolerance to a DMARD the highest tolerated dose was used and, if DAS ≥ 1.6 at the next visit, the patient went on to the next step.

Conventional care was treatment according to the treating rheumatologist's preference. The rheumatologist had access to the DAS, but was not prompted to make treatment decisions based on the DAS. In order to maintain a certain amount of homogeneity in the treatment of the conventional care group, and to maintain contrast between the groups in terms of therapy, the following order of drugs was suggested to the treating rheumatologist: hydroxychloroquine, sulfasalazine, methotrexate and leflunomide. Furthermore, the treating physician could only change therapy if DAS >2.4 at the 3 month assessment time points and after consulting the trial supervisor (DvS). During the course of the inclusion period (June 2004 – June 2007), the conventional care of RA became more aggressive in general. Therefore, from August 2005 onwards, after the inclusion of 25 patients, the treating physician was allowed to start therapy with methotrexate in the conventional care group if deemed necessary. Intra-articular corticosteroid injections were not regulated.

Assessments

Every 3 months the DAS was assessed by a research nurse. Questionnaires for physical function i.e. the Health Assessment Questionnaire (HAQ) (19) and Short Form 36 (SF-36) (20) were completed yearly. Side effects were documented and divided into adverse events or serious adverse events. Serious adverse events were defined as any adverse reaction resulting in any of the following outcomes: a life-threatening condition or death, a significant or permanent disability, a malignancy, hospitalization or prolongation of hospitalization, a congenital abnormality, or a birth defect. Radiographs of hands, wrists and feet were obtained at baseline, 1 and 2 years. All radiographs were read separately by two

experienced rheumatologists, who were unaware of the identity of the patient and of the treatment group, and the mean values were used. The radiographs were read according to time sequence and scored according to the Sharp-van der Heijde method (21). Prior to reading the trial radiographs, the two readers (DvS and PP) separately read 22 sets of radiographs of hands and feet from which an intraclass correlation coefficient of 95% was calculated. The trial physician (IvE) verified adherence to the protocol every 3 months. All protocol deviations were recorded.

Endpoints

The primary endpoint was the progression of radiographic joint damage at two years. Radiographic progression was assessed in two manners: the absolute difference in Sharp score (21;22) and the development of new erosions between baseline and two years. Erosions were diagnosed if any individual joint incorporated in the SHS score showed bone cortex disruption on radiographs of hands and/or feet in anteroposterior projection. Secondary endpoints were differences between the two treatment strategies after two years regarding DAS, the percentage of patients in clinical remission ($\text{DAS} < 1.6$), HAQ, and adverse events.

Sample size and statistical analysis

For the sample size calculation we looked at the appearance of new erosions in the first two years of treatment in patients of the early arthritis clinic of the Jan van Breemen Institute, who had 2-5 swollen joints and no erosions at their first visit, in the period before the design of the study. Since SHS scores were not available for this group, we used the radiologist's report and found a frequency of 37%. We hypothesized a frequency of new erosions of 10% in the aggressive group and calculated a sample size of 80.

Missing data for the primary and secondary endpoints was treated as follows: absence of a radiograph of hand and feet or HAQ score at two years was defined as missing. If a patient developed erosions on radiographs at one year but the two year radiographs were missing, that patient was included in the analysis as "erosion developer". If a DAS score at two years was lacking it was replaced by the DAS score of 21 months (the principle of last observation carried forward) if available, otherwise missing. All available data was included for intention-to-treat analysis. To account for loss of data of the 5 patients, who had follow-up radiographs at one year, but not at two years, we performed an additional analysis. An individual regression line of the two or three available SHS values of each included patient was calculated, and the slopes of all regression lines were compared between the groups. Data

are expressed as mean (SD) or median (range) as appropriate. Student's t-test was used to compare continuous normally distributed variables between groups. Non-parametric Mann-Whitney U tests were used when appropriate. For dichotomous variables Pearson chi-square test was used. A two-tailed probability value of $P < 0.05$ was considered statistically significant.

RESULTS

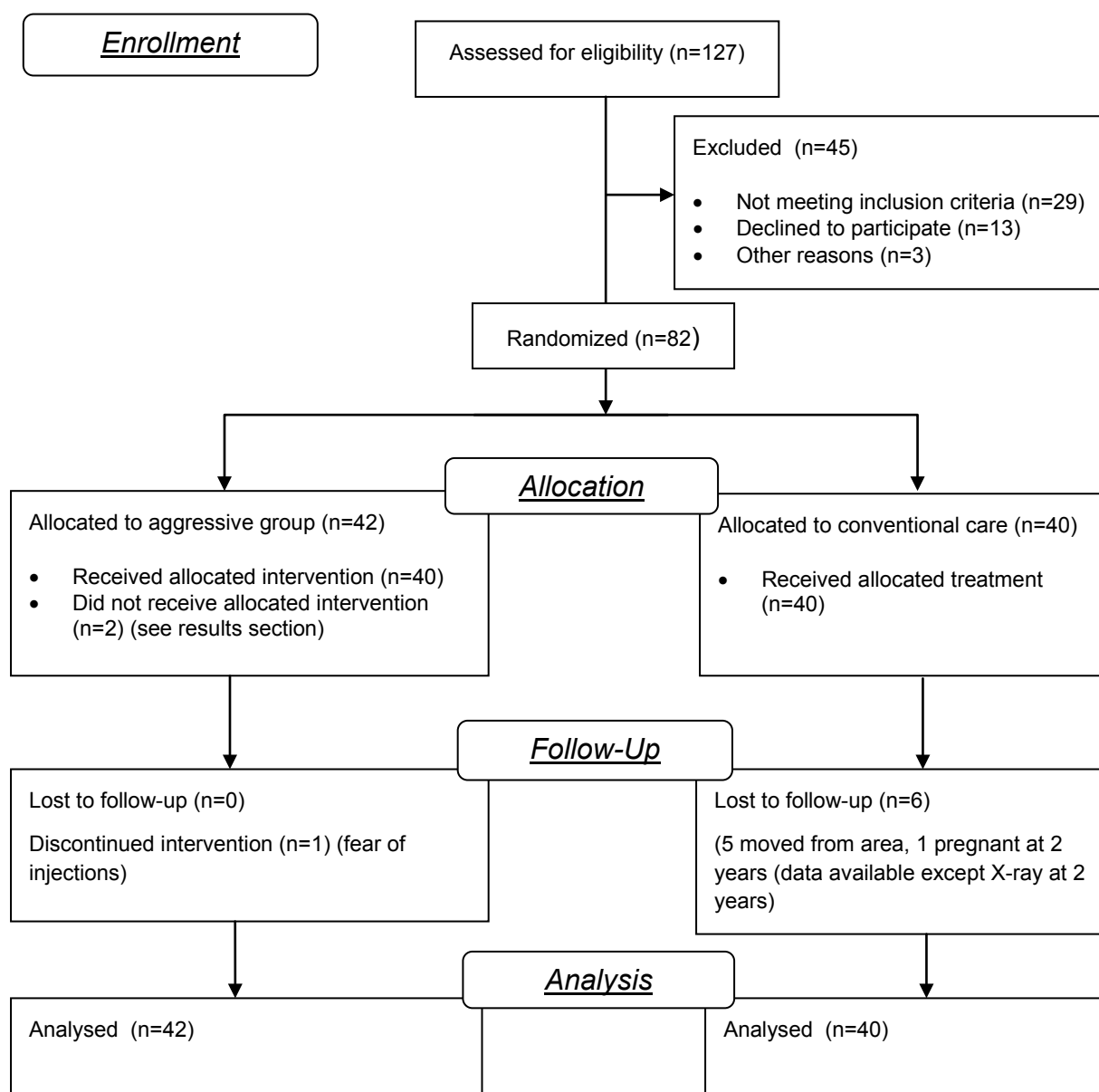
Patient characteristics

Randomization of 82 patients created generally balanced groups (aggressive group $n=42$, conventional care group $n=40$, with a mean age of 47 years and a mean symptom duration of 6 months (table 1). ACPA was present equally in both groups. In the aggressive group there was a higher percentage of RF positivity and of fulfilment of the 1987 ACR criteria for RA, whereas in the conventional care group there was a higher mean DAS and CRP. Two patients in the aggressive group discontinued adherence to the protocol within three months after randomization (but were not lost to follow-up): one patient decided directly after randomization not to take any anti-rheumatic drugs and one patient stopped taking methotrexate after an episode of fever which required hospitalization and was ascribed to methotrexate use. As this took place in the first year of the trial, 2 extra patients were randomized, both were allocated to the aggressive group, which explains why there were 42 patients in the aggressive group and 40 in the conventional care group (see also table 2).

Table 1. Baseline demographic and disease characteristics of the tight control and conventional care group

	Tight control (n=42)	Conventional care (n=40)
Age, years	48 \pm 13	46 \pm 12
Female, %	58	79
Disease duration, months	6 (3-10)	6 (4-9)
IgM-RF positive, %	48	33
ACPA positive, %	60	60
DAS	2.2 \pm 0.5	2.4 \pm 0.7
CRP (mg/l)	6 (2-10)	9 (3-21)
HAQ-score	0.50 (0.25-0.88)	0.69 (0.32-1.06)
Fulfilment of 1987 ACR criteria for RA,%	36	25
Fulfilment of 2010 criteria for RA,%	69	68

RA: rheumatoid arthritis; RF: rheumatoid factor; ACPA: anti-citrullinated protein antibody; DAS (44 joints): Disease activity score of 44 joints; CRP: C-reactive protein; HAQ: Health Assessment Questionnaire. Values are presented as mean \pm SD or median (interquartile range), as applicable.

Table 2. Consort flow diagram

Treatment

In the aggressive group, 19 patients (45%) were eventually treated with adalimumab starting after a median of 9 months (figure 1); of these 4 reached remission and 11 continued with the next step, 3 were still treated with adalimumab at two years and 1 patient did not continue adalimumab for fear of injections. One patient was treated with leflunomide starting at 15 months, and none reached the intramuscular gold step (figure 1). In the conventional care group, 24 patients started with hydroxychloroquine (subsequently 5 switched to sulfasalazine and 8 to methotrexate), 2 with sulfasalazine and 14 with methotrexate (see figure 2). The mean dose of methotrexate among methotrexate-users attained in this group was 19 mg/w. In the conventional care group a significantly higher number of patients received corticosteroid injections during follow-up (18 intra-articular and 4 intra-muscular injections in 13 patients in the conventional care group versus 7 intra-articular and 3 intra-muscular injections in two patients in the aggressive group, $p=0.001$).

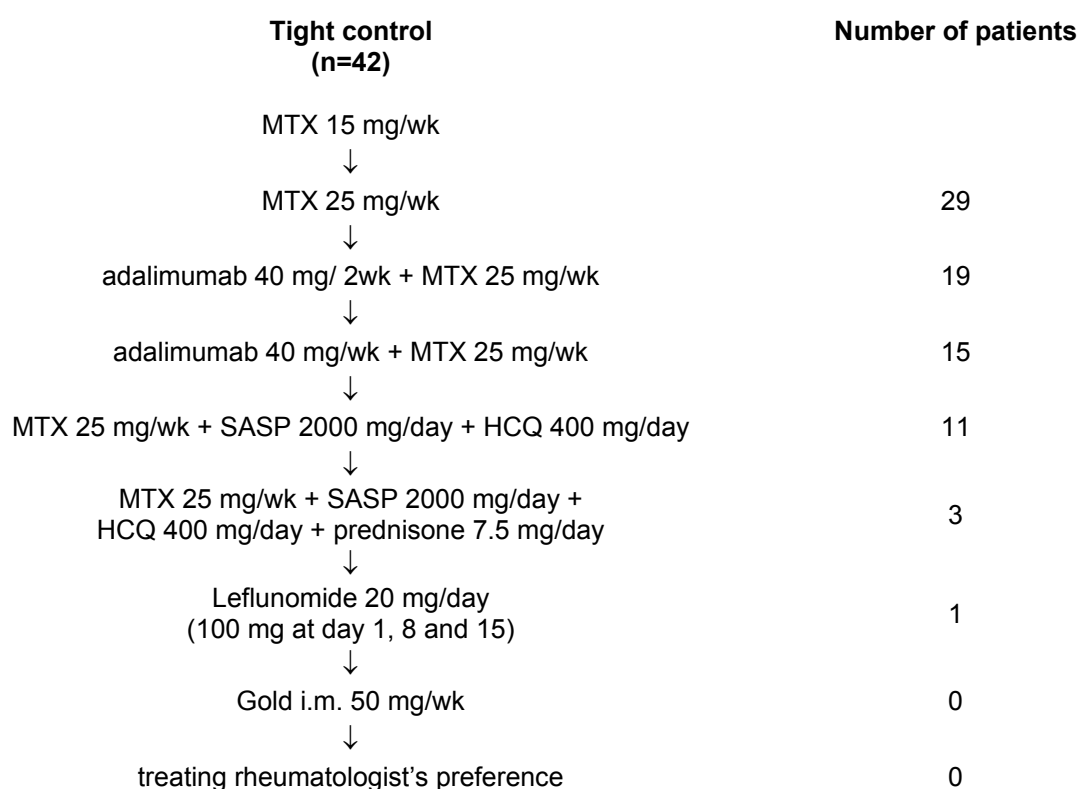


Figure 1. Flow diagram of the possible consecutive treatment steps in the tight control group Therapy was aimed at achieving and maintaining a DAS (44 joint score) of <1.6. If the DAS was ≥ 1.6 at a given time point, the therapy was changed according to this scheme. If the DAS was <1.6 at one time point the treatment remained unchanged. If the DAS was <1.6 at two consecutive time points medication was tapered. MTX=methotrexate, SASP=sulfasalazine, HCQ=hydroxychloroquine. The column on the right shows the number of patients reaching the corresponding treatment step during follow up.

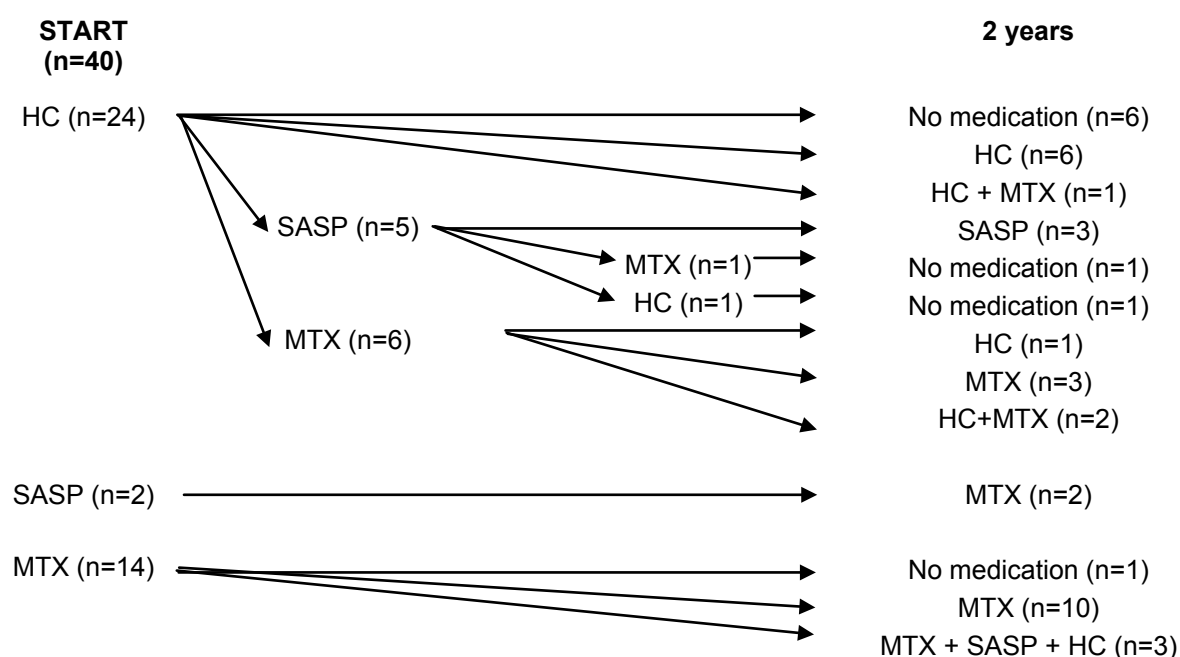


Figure 2. Medication prescribed in the conventional care group. This figure depicts the initial therapy and number of patients receiving that therapy in the conventional care group at baseline (first column), treatment steps within the trial period (second and third column) and the therapy and number of patients receiving that therapy at two years (fourth column). HC= hydroxychloroquine; SASP=sulfasalazine; MTX=methotrexate.

Radiography

Median SHS increase between 0 and 2 years was 0 (IQR 0-1.0) in the entire aggressive group and 0.25 (IQR 0-2.8) in the entire conventional care group ($p=0.19$). A cumulative probability plot of radiographic progression in the two groups is shown in figure 3. A baseline erosion with SHS<5 (thus allowing inclusion) was present in 3 patients of the aggressive group and in 6 patients of the conventional care group. New erosions developed in 5 of 39 patients (13%) starting without erosions in the aggressive group, and in 8 of 34 patients (24%) starting without erosions in the conventional care group ($p=0.25$). Data on the primary endpoint, radiographic damage, was lacking in a total of six patients at two years, all in the conventional care group. Of these, five patients dropped out (three moved out of the area and two could not be contacted anymore) and one patient was pregnant at 2 years. Of five of these patients radiography of hand and feet was available at 1 year: SHS scores at one year were 0 in two patients, 0.5 in one patient (with baseline SHS score 0), 4.5 in one patient (with baseline SHS score 2) and 8 in one patient (with baseline SHS score 4.5). Since 5 patients only had follow-up radiographs at one year, we performed an additional analysis, calculating an individual regression line of the two or three available SHS values of each patient, and then comparing the slope of the regression lines between the groups. The result remained statistically nonsignificant ($p=0.17$).

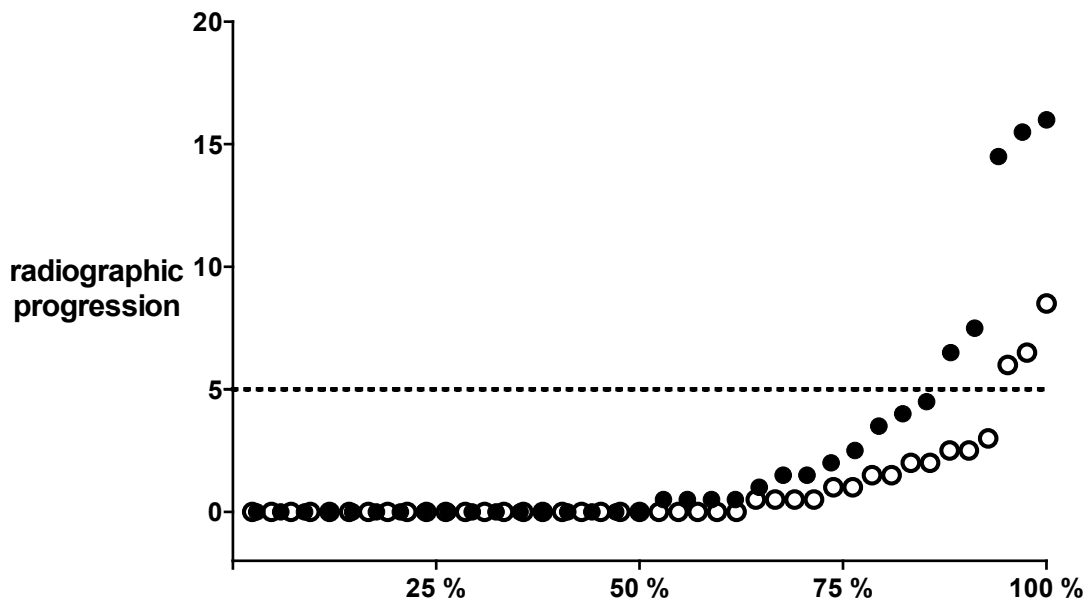


Figure 3. Cumulative probability plot of radiographic progression. Radiographic progression (Sharp/vd Heijde units) at 2 years compared to baseline in the tight control group (open dots) and conventional care group (closed dots). Every dot represents a patient. The dotted line is set at 5 Sharp/vd Heijde units as the minimal clinically important difference.

Eight patients (3 in the aggressive group and 5 in the conventional care group) had an SHS increase of 5 or more. The characteristics of this subgroup of patients compared to the patients with an SHS increase less than 5, are shown in table 3. Six of the eight patients received high-dose methotrexate (22.5-25 mg/wk), in one case also adalimumab, for most of the time. Three patients (all in the conventional care group) had an SHS increase of over 14 points. Two of them were treated with high-dose methotrexate, one from 1 year onward and one from 18 months onward. All three were in DAS remission during at least 4 of 8 measurements after baseline with a mean DAS of 1.5.

Disease activity, remission and functionality

Mean DAS in the aggressive group at 0 and 2 years was 2.2 and 1.4, respectively, and 2.4 and 1.7 in the conventional care group (figure 4 upper). Remission rates in the aggressive group at 1 and 2 years were 54 and 66%, respectively, and 65 and 49% in the conventional care group (figure 4 middle). Seven patients in the aggressive group (17.9%) and six patients in the conventional care group (15.8%) reached a period of medication free remission ($P=0.80$). The median duration of medication free remission was six months in the aggressive group and seven and a half months in the conventional care group with a range of three to nine months and three to twelve months, respectively. In the aggressive group two

out of seven patients had reactivation of disease activity after medication free remission and restarted treatment with methotrexate. In the conventional group all patients remained medication free until the end of the trial. The mean HAQ decrease at two years compared to baseline was 0.09 in the aggressive group and 0.125 in the conventional care group ($P=0.2$). (figure 4 bottom). A total of 28 protocol violations for varying reasons were recorded, all in the aggressive group. 19 violations were recorded due to not proceeding to the next treatment step with a low DAS score just above 1.6, nine violations were caused by not tapering medication after a third time DAS <1.6 . All comparisons were statistically nonsignificant, both for the primary and the secondary outcome measures.

Subgroup analysis for ACPA positive patients

Due to the growing recognition of the importance of ACPA as a prognostic marker of RA, we added a subgroup analysis including only the ACPA positive patients. In general the groups were comparable. Although median SHS scores at two years and change of SHS score between baseline and two years tended to be higher in the conventional care group, values remained low and not significantly increased compared to the aggressive group (1 (0 – 2.5) and 1.75 (0 – 4.1) ($p=0.3$) for median SHS scores at two years and (0 (0 - 2.3) and 0.75 (0 - 3.75) ($p=0.3$) for change of SHS score between baseline and two years, in the aggressive group and conventional care group, respectively).

Table 3. Baseline demographic and disease characteristics of the subgroups of patients with a delta SHS < 5 and a delta SHS ≥ 5 at two years compared to baseline

	delta SHS < 5 (n=68)	delta SHS ≥ 5 (n=8)
Age, years	48 (41-56)	36 (31-55)
Female, n (%)	47 (69)	7 (88)
Disease duration, months	6 (4-9)	7 (4-11)
IgM-RF positive, %	41	38
Anti-CCP positive, %	59	88
Disease Activity Score (44 joints)	2.3 (1.9-2.7)	2.4 (1.8–2.7)
CRP (mg/l)	8 (3-15)	7 (2-14)
HAQ-score	0.5 (0.25-0.88)	0.88 (0.13-1.0)
Fulfilment of ACR criteria for RA, %	27	50
Fulfilment of 2010 criteria for RA, %	70	88

RA: rheumatoid arthritis; RF: rheumatoid factor; ACPA: anti-citrullinated protein antibody; DAS (44 joints): Disease activity score of 44 joints; CRP: C-reactive protein; HAQ: Health Assessment Questionnaire. Values are presented as mean \pm SD or median (interquartile range), as applicable.

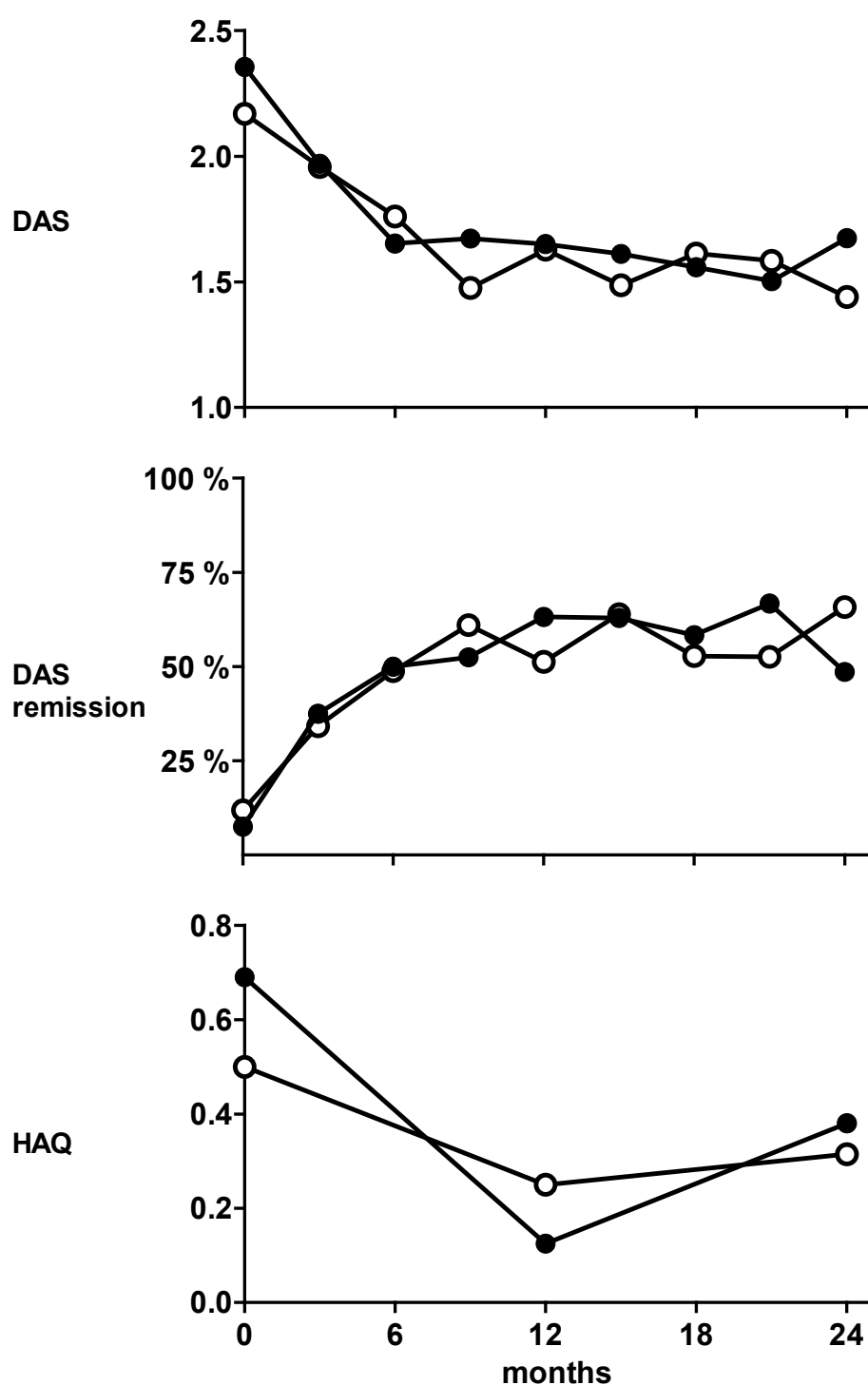


Figure 4. Secondary endpoints Disease Activity Score and Health Assessment Questionnaire in the tight control group and the conventional care group. Upper: mean disease activity scores at each time point. **Middle:** Remission rates (percentage of patients with disease activity score < 1.6) at each time point. **Bottom:** Median HAQ scores at baseline, 1 and 2 years. Open dots represent the tight control group, closed dots represent the conventional care group.

Adverse events

In the aggressive group, 59% of the patients experienced at least one adverse event during the follow up period versus 42% in the conventional care group, which was a trend ($p=0.08$). The total number of adverse events was significantly higher in the aggressive group versus the conventional care group (62 versus 35, $p=0.034$). Eight serious adverse events in seven patients were documented: five in the aggressive group and three in the conventional care group. Four were medication-related: 3 hospitalizations in the aggressive group and 1 in the conventional care group. In the aggressive group one patient was hospitalized for fever during methotrexate therapy, classified as drug-induced fever. Another patient was hospitalized twice: once for fever during adalimumab therapy and once for active RA and a rash based on acute generalized exanthematous pustulosis, attributed to adalimumab. In the conventional care group, one patient was hospitalized for gastrointestinal problems, which were attributed to methotrexate therapy.

DISCUSSION

In this trial of an “aggressive” versus a “conventional” approach to early oligoarthritis, most patients had an excellent outcome with respect to disease activity, functionality and radiographic damage regardless of the treatment group they were randomized to. A minority of patients in both groups experienced radiographic progression despite treatment with higher-dose methotrexate and despite being in remission at most time points. There was also a substantial amount of adverse events, especially in the aggressive group.

The radiological results (figure 3) give rise to the expectation that the difference between the groups would have been significant (in favour of the aggressive group), if the sample size would have been larger, thus suggesting a lack of statistical power. In addition, not all patients had radiographs at the two-year point. In our view, the main reason for the lack of statistically significant differences in the outcome parameters between the groups is that the gradual intensification of the conventional care during the course of the study including a higher amount of corticosteroid injections in that group led to less contrast in therapy between the groups and to a lower than originally expected rate of radiographic damage in the conventional care group (24% observed instead of 37% expected new erosions). Whereas the aggressive group achieved around the 10% rate of new erosions as had been assumed for the power calculation. The general trend in the treatment of (rheumatoid) arthritis is towards earlier and more aggressive treatment. At the time the study was designed in 2003, both study arms were acceptable for the participating rheumatologists. During the study, however, the conventional care needed to be intensified to accommodate

changing views, and presently, even the aggressive arm is considered to be not so aggressive anymore, since adalimumab therapy was postponed until 6 months in nonresponders. Furthermore, although ACPA positivity was equal among the groups, the study was not designed to separately analyze ACPA positive and negative subgroups, which were less prominently seen as important subgroups during the design phase of the study.

There were some drawbacks to using DAS-steered treatment aiming for remission (DAS <1.6). Three patients had significant radiographic progression, although they were in DAS-remission most of the time. As has been noted before, clinical remission is no guarantee for radiological remission (23), although a recent review showed that patients that achieve remission, defined in any way, will generally develop less radiological damage and deterioration of physical function compared to patients not reaching remission (24). Secondly, it occurred a few times during the course of the study that patients without any swollen joints would proceed to the next step of high-dose methotrexate or adalimumab, because they had a DAS of 1.6 or more due to a high value of the DAS component of patient-reported general health. In a number of cases, based on this, the treating physician refused to intensify treatment. In the light of the intensity of treatment needed to achieve these results, the number of adverse events that occurred (in both groups, but more so in the aggressive group) calls to mind the task of the physician to weigh the possible benefits of treatment against the possibility of causing harm. A related issue is the value of the DAS as a measure of remission in RA; although the DAS can be >1.6 while joints are neither swollen nor tender, the opposite also occurs: a patient reaches DAS remission in the presence of tender and/or swollen joints. In this case, one might better speak of minimal disease activity rather than remission (25). This brings up the question, 'what is real remission'? For this purpose, the ACR and EULAR have recently constituted a committee charged with the task to redefine remission in RA (26).

Other trials in early oligoarthritis or undifferentiated arthritis have noted some benefit from treatment with intramuscular or intraarticular corticosteroids compared to placebo or nonsteroidal antiinflammatory drugs (27;28), although a recent study observed that neither remission nor development of RA was delayed by intramuscular glucocorticoid treatment (29). Three months of infliximab did not prevent progression to RA (30) after 1 year, nor did abatacept monotherapy (31), although abatacept had an impact on radiographic and MRI inhibition, which was maintained for 6 months after treatment stopped. Methotrexate was successful for postponing the diagnosis of RA after 1.5 years, as well as in retarding radiological damage (15). The positive results of the latter study were confined to the subgroup of ACPA-positive patients. In these trials, adverse events were generally not a problem.

Since the present study has not demonstrated a functional or radiological benefit of aggressive over conventional treatment, we cannot recommend aggressive therapy in all patients presenting with two to five swollen joints. The benefit of aggressive treatment in early inflammatory arthritis is not as evident as it is in polyarthritis, and many patients achieved good results including prevention of erosive disease with hydroxychloroquine only, as has been found before in early RA (16;32). The treatment of oligoarthritis should ideally depend on an accurate prediction of prognosis. The most important prognostic factors are ACPA and radiographic damage (33). These data combined with the results of the present study may suggest that in patients with early inflammatory arthritis a radiographic-driven therapy may be superior to the widely used DAS-driven therapy in reducing structural joint damage, but more research is needed to further refine prediction and thus guide therapy at the individual level.

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Chapter 3.2

Circulating microparticles remain associated with complement activation despite intensive anti-inflammatory therapy in early rheumatoid arthritis

I.C. van Eijk
M.E. Tushuizen
A. Sturk
B.A.C. Dijkmans
M. Boers
A.E. Voskuyl
M. Diamant
G.J. Wolbink
R. Nieuwland
M.T. Nurmohamed

ABSTRACT

Objectives. Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease characterized by synovitis and joint destruction. The pathogenesis of RA is not clear, but is considered to be an immune-mediated inflammatory disorder, in which the complement system plays an important role. Although cell-derived microparticles (MP) have been associated with inflammation and complement activation, it is unknown whether MP are either cause or consequence. Therefore, we investigated whether circulating MP differ between very early yet untreated arthritis patients and healthy controls, and whether intensive anti-inflammatory treatment of such patients affects circulating MP.

Methods. RA patients (n=24) and controls (n=15) were included. Nine RA patients were re-evaluated after 8 weeks of intensive treatment with a combination of drugs (COBRA-scheme). Disease activity was measured by ESR, C-reactive protein (CRP) and disease activity score of 28 joints (DAS28). Flow cytometry was used to study MP and exposure of complement activator molecules and complement components.

Results. At baseline, concentrations of MP exposing C1q, CRP or SAP were all significantly elevated in early RA patients compared to controls ($p=0.003$, $p=0.002$, and $p=0.003$, respectively). Upon treatment, DAS28, ESR and CRP significantly decreased ($P=0.008$, $P=0.008$ and $P=0.012$), but the concentrations of circulating MP and MP exposing complement components or activator molecules were unaffected.

Conclusions. Circulating MP exposing complement components or activator molecules are elevated in early RA. Since a strong anti-inflammatory therapy suppressed inflammation in early RA patients but not levels of circulating MP, it is unlikely that inflammation is the main underlying cause of MP release in these patients.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease with a complex pathogenesis, characterized by synovitis leading to cartilage, tendon and joint destruction (1;2). Although the pathogenesis of RA is not clear, it is considered to be an immune mediated inflammatory disorder, in which the complement system plays an important role.

Recently, cell-derived microparticles (MP), which are small membrane vesicles released from blood cells or endothelial cells upon activation or during apoptosis, were shown to be associated with complement activation, inflammation and coagulation in various diseases, including inflammatory diseases (3-7). Although inflammation causes release of MP and in turn MP may induce or enhance inflammation, it remains unknown whether circulating MP merely reflect ongoing inflammation or whether MP actually contribute to the disease development.

In vitro studies and animal models indicate that inflammatory mediators such as TNF α and interleukin (IL)-1 trigger MP release (8-10). Several other observations, however, suggest a more active role for MP in development of rheumatoid inflammatory activity. Leukocyte-derived MP, present in synovial fluid of RA patients, not only expose tissue factor and trigger coagulation (11), but also induce the production and release of chemokines and cytokines by fibroblast-like synoviocytes, which in turn may further contribute to synovial inflammation and angiogenesis (12). Circulating platelet-derived MP (PMP) were reported to be elevated in RA patients, compared to controls, and these PMP were associated with disease activity as measured by the disease activity score of 28 joints (DAS28) (3). Finally, synovial MP may also be involved in complement activation in RA patients, since we recently demonstrated the presence of bound complement components C1q, C3 and C4 as well as complement activator molecules on circulating MP from RA patients, further supporting their role in complement activation (4).

To determine whether circulating MP numbers are associated with inflammatory activity in RA patients, we compared MP in very early yet untreated arthritis patients and healthy controls. Additionally, we determined the effects of changes in disease activity upon intense anti-inflammatory therapy with the COBRA strategy (13) on MP numbers and composition.

MATERIALS AND METHODS

Patients

Consecutive untreated RA patients (n=24) were included and venous blood was collected at baseline in the fasting state. Of these patients, 9 were enrolled in a trial addressing the

effects of tight control and intensified COBRA combination treatment in early RA and were treated with COBRA treatment comprising sulfasalazine, methotrexate and high-dose step-down prednisolone (60 mg/day (week 1); 40 mg/day (week 2); 30 mg/day (week 3); 20 mg/day (week 4); 15 mg/day (week 5); 10 mg/day (week 6); 7.5 mg/day thereafter) (14). Criteria for inclusion in that study were active disease defined by a DAS28 score > 3.2. Of these 9 patients, an additional fasting blood sample was collected after 8 weeks of treatment. Furthermore, fasting blood was collected from healthy age matched controls (n=15). All patients fulfilled the criteria of the American College of Rheumatology for RA (15). All participants gave written informed consent and the study protocol was approved by the Institutional Ethics Committee of the Slotervaart Hospital, Jan van Breemen Institute and BovenIJ Hospital.

Collection of blood samples

Subjects were asked to refrain from beverages other than water (particularly no caffeine containing beverages or alcohol), smoking, medication and meals from midnight prior to the testing day. Blood was collected from the antecubital vein in tubes containing 0.5 mL 3.2% sodium citrate (BD, San Jose, CA). Cells were removed by centrifugation (20 minutes at 1550g and 20°C) within 10 minutes after collection. Aliquots of cell-free plasma (250 µL) were snap-frozen in liquid nitrogen for at least 15 minutes and stored at -80 °C (16).

Isolation of MP

MP were isolated from plasma aliquots (250 µL) after thawing on melting ice by centrifugation (30 minutes at 18,890g and 20 °C). After centrifugation, MP-free supernatant (225 µL) was removed. The remaining MP pellet was washed with 225 µL phosphate-buffered saline (PBS) containing (0.32% w/v) trisodium-citrate (pH 7.4). After centrifugation, the supernatant was removed and the MP pellet was resuspended in PBS-citrate (75 µL).

Labelling of MP

Aliquots of MP (5 µL) were diluted in 35 µL of PBS containing 2.5 mmol/L CaCl₂ (PBS/Ca, pH 7.4). Subsequently, 5 µL allophycocyanin (APC)-labeled annexinV (Caltag Laboratories, Carlsbad, CA) was added and combined with either fluorescein isothiocyanate (FITC)-labeled CD61 (DakoCytomation, Glostrup; Denmark) plus phycoerythrin (PE)-labeled CD62p (P-selectin) or CD63 (glycoprotein 55; both antibodies from Immunotech, Fullerton, CA), or FITC-labeled CD144 (Alexis, San Diego, USA) plus E-selectin (CD62e-PE; Ancell, Bayport, MN). For appropriate settings of fluorescence thresholds, MP were incubated with isotype-matched control antibodies, i.e. PE- labeled IgG₁ and/or FITC-labeled IgG₁ (BD, San Jose, CA), or FITC-labeled Ig (IQP, Groningen, the Netherlands). MP were labelled for 15 minutes at room temperature, and labelling was stopped by addition of PBS/calcium (900 µL) to each

tube. Samples were analyzed for one minute on a FACS Calibur (BD) and data were analyzed using Cellquest Pro (version 4.0.2; BD) (17).

Alternatively, MP (5 μ L aliquots) were incubated for 30 minutes at room temperature with anti-C1q, anti-C3-15, anti-CRP 5G4, anti-SAP-14, anti-IgM, anti-IgG (gift from Sanquin; Amsterdam, the Netherlands) or isotype-matched control antibodies IgG₁ and IgG_{2a} (Pharmacia, Montlingen, Switzerland) in a final volume of 50 μ L of PBS containing 2.5 mmol/L CaCl₂ (PBS/Ca, pH 7.4). After labeling, MP were washed with PBS/calcium (200 μ L). Subsequently, PE-labeled F(ab')₂ and APC-labeled annexinV were added and the mixtures were incubated for 30 minutes at room temperature. For setting of fluorescence thresholds, MP were incubated with isotype-matched control antibodies, i.e. PE- labeled IgG₁ and/or FITC-labeled IgG₁ (BD), or FITC-labeled Ig (IQP). Finally, PBS/calcium (400 μ L) was added to each tube and samples were analyzed for one minute on a FACS Calibur (BD). Data were analyzed using Cellquest Pro (version 4.0.2; BD) (6). All antibodies and control antibodies used were tested and titrated using purified cells and MP before use.

Identification and characterization of microparticles

MP were defined according to size (forward scatter), side scatter and binding of annexin V, a protein that binds with high affinity and specificity to phosphatidylserine, as described previously (18). It should be mentioned, that the percentage of microparticles binding annexin V increases by centrifugation and freeze-thawing. Under these conditions binding of annexin should be considered as a marker to identify microparticles rather than reflecting the exposure of negatively charged phospholipids such as phosphatidylserine. The within-run coefficient of variation (CV) is 8% and the day-to-day CV is 13%. The presence of bound complement components (C1q, C3 and C4) as well as bound adapter molecules (CRP, SAP, IgM and IgG) was studied using flowcytometry as described earlier (4).

Statistical analysis

Data were analyzed with SPSS for Windows 16.0 (SPSS Inc). According to their distribution, the various parameters are expressed as mean (\pm standard deviation) or median (interquartile range). Data with a non-Gaussian distribution was log transformed for analysis if possible. To compare the groups, student's T-test or Mann-Whitney U test was used when appropriate. Furthermore, correlations between variables were analyzed by using Pearson correlation or Spearman's rho tests. Univariate linear regression analyses were performed on log-transformed data to investigate the influence of possible confounders (i.e. sex, smoking status, systolic blood pressure and body mass index (BMI) on the results. Wilcoxon signed-rank test was used to investigate the differences in values at baseline and at 8 weeks in the

prospectively followed subgroup of patients (n=9). P-values less than 0.05 were considered statistically significant.

RESULTS

Characteristics and inflammatory measures

Baseline demographic and clinical characteristics of the RA patients are summarized in Table 1. The majority of RA patients was IgM-rheumatoid factor and/or anti-citrullinated protein antibody (ACPA) positive. Their DAS28 score (mean 5.2) reflects patients with a high disease activity. Both ESR and CRP levels were significantly elevated in patients compared to controls. The patient group comprised fewer females and had higher systolic blood pressure than the controls. The nine patients prospectively followed had similar age, BMI, systolic blood pressure and DAS28 scores, but higher CRP (P=0.04) and ESR (p=0.06) levels compared to the other RA patients (n=15; see Table 1 and Table 3).

Table 1. Baseline characteristics of the RA patients and healthy controls

	Controls (n=15)	Patients (n=24)	P
Age, years	49 ± 11	51 ± 11	0.91
Female, n (%)	13 (87)	14 (58)	0.02
RF positive, n (%)	N/A	16 (67)	N/A
Anti-CCP, n (%)	N/A	17 (71)	N/A
DAS28	N/A	5.2 ± 1.3	N/A
ESR	5 (3-8)	33 (15-48)	<0.001
CRP	1 (1-2)	13 (3 – 37)	<0.001
NSAID use, n (%)	N/A	17 (77%)	N/A
Smoking, %	7	30	0.13
Systolic blood pressure (mm Hg)	119 ± 7.8	131 ± 22.8	0.03
Diastolic blood pressure (mm Hg)	79 ± 5.9	79 ± 10.8	0.93
BMI	23.4 ± 2.0	25.1 ± 4.3	0.15

RA: rheumatoid arthritis; RF: rheumatoid factor; Anti-CCP: anti-cyclic citrullinated peptide; DAS28: Disease activity score of 28 joints; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; NSAID: non steroidal anti-inflammatory drugs; BMI: body mass index (weight in kg/length in m²). Values are presented as mean ± SD or median (interquartile range), as applicable.

Elevated concentrations of MP exposing complement components or activator molecules in early RA

The total number of MP did not differ between patients and controls (Table 2). In patients, the number of MP exposing C1q, CRP and SAP were significantly elevated compared to controls (Table 2 and Figure 1). These results remained unchanged after adjusting for possible confounders (data not shown). At baseline, ESR and CRP significantly correlated with MP exposing C1q, CRP and SAP (for ESR: $r=0.37$, $P=0.02$; $r=0.54$, $P<0.001$ and $r=0.46$, $P=0.003$, respectively and for CRP: $r=0.39$, $P=0.02$; $r=0.52$, $P=0.001$ and $r=0.36$, $P=0.02$, respectively), confirming the association between ongoing inflammation and circulating MP.

Table 2. Complement component and activator molecules exposing MP in patients and controls

MP and complement components	Controls (n=15)	Patients (n=24)	P
Total MP	103.6 (64.5 – 129.9)	108.9 (70.4 – 185.0)	0.27
C1q	4.7 (1.7 – 8.8)	9.4 (5.5 – 14.2)	0.003
C4	11.6 (7.0 – 15.8)	17.8 (7.6 – 25.0)	0.35
C3	11.3 (7.7 – 15.0)	9.1 (2.5 – 19.5)	0.34
CRP	3.0 (1.3 – 4.6)	7.3 (2.5 – 19.5)	0.002
SAP	47.9 (22.9 – 59.5)	95.3 (62.0 – 155.6)	0.003
IgM	18.4 (11.5 – 34.2)	24.4 (14.4 – 42.3)	0.43
IgG	3.6 (1.0 – 6.6)	3.3 (2.0 – 6.5)	0.64

Values are presented as median (interquartile range (IQR)); MP: numbers $\times 10^4/\text{mL}$; P values were calculated using Mann-Whitney U test; CRP, C-reactive protein; SAP, Serum amyloid-P.

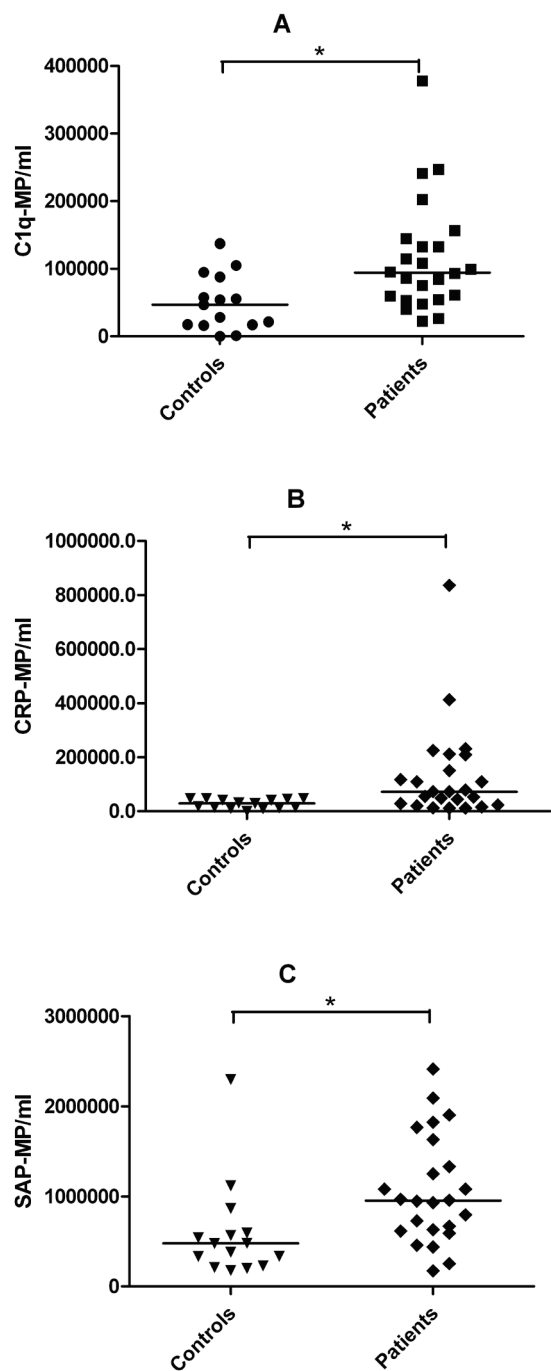


Figure 1. Concentration of circulating microparticles (MP) exposing C1q (A), CRP (B) or serum amyloid-P (SAP; C) in plasma of RA patients and healthy individuals. Individual values are shown, with the horizontal lines representing the median. Differences were analyzed with Mann-Whitney U test. *P<0.001

Intense inflammatory suppression does not alter MP composition

Upon treatment with intense anti-inflammatory therapy, DAS28, ESR and CRP decreased significantly (Table 3), but the concentrations of total circulating MP and MP exposing complement components or activator molecules were unaffected (Table 3, Figure 2). Numbers of MP exposing C1q or CRP were still significantly elevated in the RA patients after treatment compared to controls (data not shown). At 8 weeks we did not find correlations between DAS28 and CRP with total MP numbers or MP exposing complement components or activator molecules.

Table 3. Inflammatory markers, complement component and activator molecules exposing MP in a subgroup of patients treated with anti-inflammatory drugs at baseline and after 8 weeks

Disease scores and MP	Baseline (n=9)	8 weeks	P
DAS28	5.2 ± 0.7	2.2 ± 1.2	0.008
ESR	45 (17 – 62)	12 (6 – 27)	0.008
CRP	19 (9 – 69)	4 (1 – 8)	0.008
Total MP	167 (73 – 239)	106.7 (36.9 – 266)	0.401
MP + C1q	14.4 (8.4 – 24.4)	13.4 (4.2 – 39.8)	0.889
MP + C4	22.2 (5.9 – 27.9)	19.1 (7.8 – 58.3)	1.0
MP + C3	10.3 (4.1 – 21.4)	12.3 (1.9 – 37.8)	1.0
MP + CRP	7.9 (3.4 – 31.2)	7.1 (2.9 – 28.1)	0.575
MP + SAP	108.2 (53.4 – 172.8)	109.3 (29.7 – 214)	0.779
MP + IgM	31.6 (13.7 – 41.4)	34.1 (15.1 – 63.1)	0.401
MP + IgG	2.3 (0.3 – 6.0)	1.2 (0.0 – 3.2)	0.674

Values are median (interquartile range (IQR)). P values were calculated using Wilcoxon signed-rank test. DAS28: disease activity score of 28 joints; MP: numbers x 10⁴/mL; CRP: C reactive protein; ESR: erythrocyte sedimentation rate; SAP: serum amyloid P. After total MP, the other categories refer to the number of MP exposing a certain complement component or activator molecules

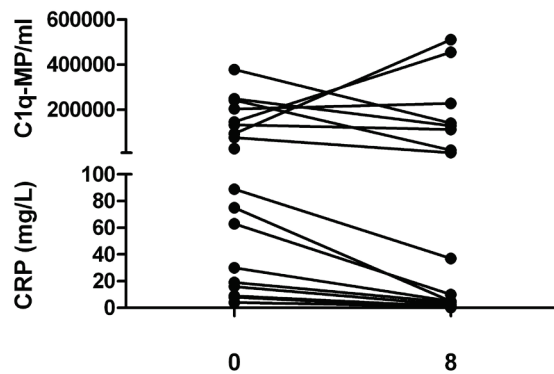
DISCUSSION

This study demonstrates that MP exposing complement components (C1q) or activator molecules (CRP or SAP) are elevated in early active RA. Although a strong anti-inflammatory therapy using a combination of DMARDs combined with high dose prednisolon (COBRA scheme (14)) strongly suppressed inflammatory activity, circulating MP were unaffected. Our present data may suggest that inflammation is not the underlying cause of MP generation in these patients.

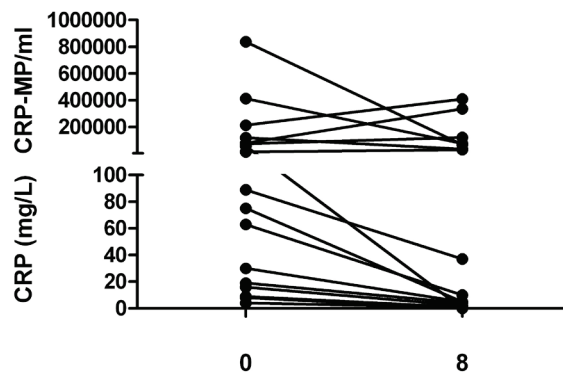
Imaging studies have shown that synovitis is still apparent in the majority of RA patients that are clinically in remission, indicating subclinical ongoing inflammation (19). The present findings suggest that MP may be one of the factors that are actively involved in this sustained inflammation in RA. We cannot exclude, however, that a delay exists between normalization

of systemic inflammation and circulating MP or their composition. Alternatively, subpopulations of circulating MP may be affected by normalization of inflammation rather than the total population of MP, as assessed in the present study. The biological relevance of MP or subpopulations thereof to the pathology of early active RA may be questioned given the fact that inflammation and disease activity were both efficiently suppressed.

C1q-MP and serum CRP at baseline and after 8 weeks



CRP-MP and serum CRP at baseline and after 8 weeks



SAP-MP and serum CRP at baseline and after 8 weeks

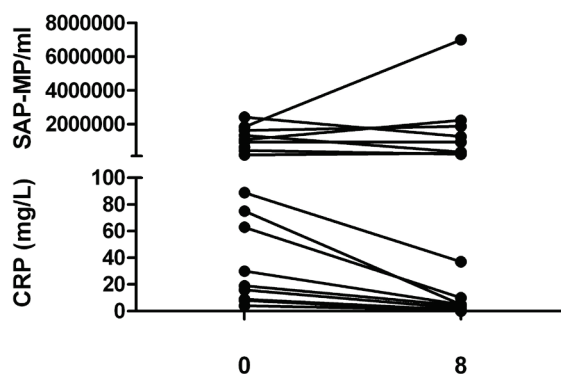


Figure 2. Depicted are the values for serum CRP and concentrations of microparticles (MP) exposing C1q (A), CRP (B) or serum amyloid-P (SAP; C) in plasma of RA patients (n=9) at baseline and after 8 weeks of COBRA treatment. Lines connect individual values at both timepoints.

Recently, MP have emerged as a new pro-inflammatory mediator. In fact, they are thought to amplify or disseminate inflammation. MP are thought to trigger inflammation by several processes such as activation of endothelial cells and leukocytes, triggering production and release of chemokines and cytokines, and by activating the complement cascade which is thought to play a key role in the pathogenesis of RA (4;12;20-24).

On the other hand, inflammation may trigger MP formation. For instance, in vitro studies showed that MP are released from cells incubated with TNF α or IL-1, and a study in mice showed that the number of platelet-derived MP in plasma markedly increased upon injection with TNF α (8-10). Data from the present study, however, implicate that MP remain associated with complement activation in early RA despite aggressive anti-inflammatory therapy.

We can not answer the question yet whether these MP really have pro-inflammatory properties and thus actively contribute to complement activation, or whether they merely reflect ongoing complement activation. Already in the 1980's, Sims and coworkers demonstrated that cells were protected from complement-induced lysis by the release of complement complex-enriched MP (25). Thus, the presence of elevated concentrations of complement-enriched MP in early RA may also be a reflection of ongoing and uncontrolled activation of the complement system.

Our main finding that powerful inhibition of inflammation did reduce disease activity but did not disturb the association between circulating MP and complement activation, suggests that both MP and complement contribute to the development of and/or the chronic character of inflammatory diseases such as RA.

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SUMMARY AND DISCUSSION

SCOPE OF THIS THESIS

In this thesis effects of anti-inflammatory treatment on cardiovascular risk factors in patients with rheumatoid arthritis (RA) or ankylosing spondylitis (AS) are investigated against the background of the intertwined mechanisms involved in inflammation and atherosclerosis. We have studied traditional cardiovascular risk factors such as dyslipidemia, insulin resistance and thyroid dysfunction. Additionally, other factors potentially influencing or mediating cardiovascular risk, such as microvascular function, surrogate markers of cardiovascular disease, such as carotid intima media thickness and possible new markers for monitoring disease activity in AS are examined. Eventually, since the increased risk of cardiovascular disease in rheumatic diseases also encourages more prompt and intensive treatment of the disease itself, the effects of tight control therapy is evaluated in an early inflammatory arthritis cohort with mild disease activity. Finally, the role of cell derived microparticles in the inflammatory process in early RA is explored.

CARDIOVASCULAR RISK AND MONITORING OF DISEASE ACTIVITY IN PATIENTS WITH ANKYLOSING SPONDYLITIS

Although literature about cardiovascular risk in AS is limited, inflammation in AS seems to act independently or synergistically with other cardiovascular risk factors in the pathogenesis of atherosclerosis. There is increasing evidence that early functional and structural vascular abnormalities (surrogate markers) such as endothelial dysfunction, intima media thickness and arterial stiffness predict cardiovascular risk independently of “classical” cardiovascular risk factors.

In **chapters 1.1 and 1.2** we observed impairment of different surrogate markers in patients with active AS. Microvascular endothelium-dependent vasodilatation, capillary recruitment in skin, as well as intima media thickness of the carotid artery were impaired as compared to control subjects. These differences remained after adjustment for established cardiovascular risk factors, which supports a potential role of these markers as a tool for identifying patients at elevated cardiovascular risk. In addition, microvascular function as well as capillary recruitment improved after TNF blocking therapy, which supports a role for inflammation deteriorating the microcirculation.

One of the most established risk factors for atherosclerosis is dyslipidemia. Inflammation deteriorates the lipid profile, which is also observed in several inflammatory rheumatic diseases. However, besides or even beyond focusing solely on lipid levels, in particular HDL-c levels, the actual composition of the HDL and thereby its functional characteristics seems to be important, in order to learn more about its effects on the vascular system and

cardiovascular risk. Nowadays, HDL protein profiling is increasingly used to determine the biochemical composition of HDL. In **chapter 1.3** we demonstrate, that during anti-TNF treatment for AS, along with favourable changes in lipid profile, HDL composition is actually altered. Serum amyloid A (SAA), which is an acute phase reactant, transported mainly in HDL as an apolipoprotein and is associated with increased cardiovascular risk, was present on HDL during active disease. However, after anti-TNF treatment SAA disappeared from the HDL particle, rendering it more atheroprotective. This observation underlines the importance of understanding the role of functional characteristics of HDL cholesterol in cardiovascular diseases, related to chronic inflammatory conditions such as AS.

Disease activity in AS is generally measured with the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI). Despite the fact that the BASDAI is a validated instrument used in many clinical trials as an outcome parameter for disease activity, it remains a subjective parameter that is based on a patient questionnaire. In recent years TNF blocking therapy has become widely available for patients with active AS. Since anti-TNF therapy is not without risks and is also very costly, it is of great importance to identify patients likely to (non)respond to this type of drug. Similar as in RA, there is need for more objective disease activity markers such as ESR and CRP. In AS, however, the sensitivity of these inflammatory markers as biomarkers of disease activity is controversial. Therefore, in **chapter 1.4**, we explored the usefulness of ESR, CRP, hsCRP, and SAA for monitoring inflammation in AS patients treated with anti-TNF along with the association between these inflammatory markers and the BASDAI over time. The data demonstrated that, unlike BASDAI, in particular CRP and SAA, served as a powerful tool not only for monitoring the efficacy of anti-TNF therapy, but also for the selection of AS patients with a high likelihood of responding to anti-TNF treatment.

CARDIOVASCULAR RISK IN PATIENTS WITH RHEUMATOID ARTHRITIS

Cardiovascular disease has been recognized as the major cause of excess morbidity and mortality in patients with RA. This increased cardiovascular risk in RA may partly be due to traditional cardiovascular risk factors, i.e. an atherogenic lipid profile and hypertension, but chronic inflammation is also thought to be important.

The "metabolic syndrome" is the co-occurrence of metabolic risk factors for both type 2 diabetes and cardiovascular disease (abdominal obesity, hyperglycemia, dyslipidemia (low HDL cholesterol and high triglycerides (TG) levels), and hypertension) and appears to be more prevalent in patients with RA compared to controls. The presence of the metabolic syndrome increases the risk for a future cardiovascular event. Another disease in which a

relationship with metabolic syndrome has been described is hypothyroidism. Hypothyroidism is a well established risk factor for cardiovascular disease and a common comorbidity in RA. Recent studies demonstrated that hypothyroidism is associated with cardiovascular disease in RA. Therefore we hypothesized that the prevalence of metabolic syndrome in patients with both RA and hypothyroidism could well be increased. Indeed, in **chapter 2.1** it is demonstrated that the prevalence of metabolic syndrome in hypothyroid RA patients is substantially elevated compared to patients with RA alone. Since having metabolic syndrome increases the risk of a cardiovascular event, this may be a possible explanation for the observation that RA with concomitant hypothyroidism are at even more pronounced cardiovascular risk than patients with RA alone.

Another important mechanism at the basis of the development of atherosclerosis is endothelial dysfunction. Microvascular endothelial dysfunction is important not only in the development of target-organ damage in the heart and kidney, but also in the development of cardiovascular risk factors such as hypertension and insulin resistance. In longstanding RA impaired microvascular function has been previously demonstrated. An intriguing question is at what point in the inflammatory disease course abnormalities in (micro)vascular function occur. In **chapter 2.2** we study microvascular function in early DMARD-naïve RA-patients with low systemic inflammatory activity, since it was unknown whether microvascular dysfunction is already present in very early stages of the disease. We observed a preserved microvascular endothelium-dependent vasodilatation and capillary recruitment during reactive hyperemia in this patient group, suggesting that systemic inflammatory activity is necessary to cause microvascular dysfunction.

In chapter 2.3 we describe two cases of RA patients with concomitant diabetes in which glycaemic control parameters beneficially changed after initiation of anti-TNF therapy. Beneficial clinical effects of treatment of RA with TNF α -antagonists on concomitant diabetes had not been previously described. TNF α had been closely linked to obesity and insulin resistance and it was suggested that TNF α -antagonists, in addition to their known powerful anti-inflammatory effects, may have a beneficial effect on insulin resistance in rheumatic diseases. Although our results are observational, the findings presented in this chapter suggest that TNF α -blockade causes better glycaemic control in RA patients with concomitant diabetes, probably by improving insulin resistance.

EARLY INFLAMMATORY ARTHRITIS

Early and aggressive treatment of patients with RA has increasingly been shown successful, particularly with combinations of disease modifying antirheumatic drugs (DMARD), also

containing anti-TNF therapy. Among the results are percentages of sustained (drug-free) remission of around 40%, excellent functional status and nearly complete arrest of radiological damage progression. In an attempt to explain these better results than had been attained before in RA of longer duration, the concept of a “window of opportunity” was proposed, suggesting that early suppression of active inflammation produces long-term benefits. **Chapter 3.1** Investigates whether the approach of striking fast and hard, which has been shown useful in active RA, is also effective in arthritis patients presenting with only moderately active disease, i.e. in those patients who would not meet the usual inclusion criteria for trials in active RA. In this randomized trial of a “fast and hard” versus a “go low, go slow” approach to early oligoarthritis, most patients had an excellent outcome with respect to disease activity, with remission rates exceeding 50%, functionality and radiographic damage regardless of the treatment group they were randomized to. Based on this data we cannot recommend aggressive therapy in all patients presenting with two to five swollen joints. This study shows that the benefit of tight control treatment in early inflammatory arthritis is not as evident as it is in polyarthritis. Since a minority of patients in both groups experienced radiographic progression despite treatment with higher-dose methotrexate and despite being in remission at most time points it may be suggested that the widely used DAS-driven therapy in reducing structural joint damage may be less useful than for example a radiographic-driven therapy.

Recently, cell derived microparticles (MP) have emerged as a new pro-inflammatory mediator. MP were shown to be associated with complement activation, inflammation and coagulation in various diseases, including inflammatory diseases. In fact, they are thought to amplify or disseminate inflammation. MP probably trigger inflammation by several processes such as activation of endothelial cells and leukocytes, triggering production and release of chemokines and cytokines, and by activating the complement cascade which is thought to play a key role in the pathogenesis of RA. On the other hand, inflammation may trigger MP formation. For instance, in vitro studies showed that MP are released from cells incubated with TNF α or IL-1, and a study in mice showed that the number of platelet-derived MP in plasma markedly increased upon injection with TNF α . However, although inflammation causes release of MP and in turn MP may induce or enhance inflammation, it remains unknown whether circulating MP merely reflect ongoing inflammation or whether MP actually contribute to the disease development. In **chapter 3.2** we determine whether circulating MP numbers are associated with inflammatory activity in RA patients, by comparing MP in very early yet untreated arthritis patients and healthy controls. Additionally, we determined the effects of changes in disease activity upon intense anti-inflammatory therapy with the COBRA strategy on MP numbers and composition. This study demonstrates that MP

exposing complement components (C1q) or activator molecules (CRP or SAP) are elevated in early active RA. Although the earlier mentioned strong anti-inflammatory combination DMARD therapy strongly suppressed inflammatory activity, circulating MP were unaffected. These observations may suggest that inflammation is not the underlying cause of MP generation in these patients. Our main finding that powerful inhibition of inflammation did reduce disease activity but did not disturb the association between circulating MP and complement activation, suggests that both MP and complement actively contribute to the development of and/or the chronic character of inflammatory diseases such as RA

GENERALISIBILITY

This thesis contributes to the concept that inflammatory diseases such as RA and AS increase the risk of cardiovascular disease. By demonstrating that inflammation deteriorates lipid profile, and that hypothyroidism elevates the risk of metabolic syndrome in RA patients, this thesis shows that the worsening of cardiovascular risk is , at least partially, caused by deterioration of traditional risk factors. In addition this thesis addresses the possibility of a direct pro-atherogenic effect of inflammation, the key feature of rheumatic diseases, on cardiovascular risk. Harmful effects of inflammation are supported by demonstrating impaired microvascular function, increased intima media thickness and increased arterial stiffness in patients with AS. Moreover signs of impaired glucose handling in patients with RA, partly reversible by anti-inflammatory therapy, are demonstrated.

Our findings may have implications for screening and management of cardiovascular comorbidity in patients with inflammatory diseases. Their increased risk should encourage active treatment for the rheumatic disease itself, since lowering the inflammatory activity seems to slow down the progression of atherosclerosis. This thesis contributes to this notion by testing an aggressive treatment strategy in a very early stage of the inflammatory arthritis.

The data show that results of both tight-control therapy and conventional therapy are excellent. However, it also shows that, until now, full disease control including radiographic arrest in all patients remains an elusive target even in moderately active early arthritis. The fact that the majority of the patients with radiographic progression were in DAS remission most of the time points out that especially in early and less active arthritis patients other markers may be preferred to monitor disease activity.

FURTHER RESEARCH

Inflammation may well be the missing link between inflammatory rheumatic conditions and excess cardiovascular risk. Future research should further elucidate the mechanisms by which such inflammation enhances cardiovascular risk. Some studies report associations between cardiovascular surrogate markers and time-specific inflammatory parameters, but not with chronic inflammatory parameters, whilst other studies report opposite results. Hence, it is interesting to investigate whether the contribution of ‘acute’ versus ‘chronic’ inflammation to cardiovascular risk is different. In addition, an intriguing question is if, and if so at what stages of the disease, the adverse effects of inflammation on the vascular system are still reversible. Observational studies demonstrate that arthritis patients, who respond to effective anti-inflammatory therapy (i.e. TNF blockade or methotrexate therapy), are associated with both a lower cardiovascular risk and improved cardiovascular risk profile. These observations strongly support further research in this area to unravel pathways through which inflammation mediates cardiovascular disease and effective anti-inflammatory drugs may have (anti-) atherogenic effects. Taken together, appreciation of shared inflammatory mechanisms in atherosclerosis and chronic inflammatory conditions, like inflammatory arthritis, is mandatory for better understanding of the cardiovascular burden in this population.

Regarding the early arthritis trial presented in this thesis, in which a minority of patients in both inflammatory arthritis groups experienced radiographic progression despite treatment with higher-dose methotrexate and despite being in remission at most time points, it may be suggested that the widely used DAS-driven therapy in reducing structural joint damage may be less useful than for example a radiographic-driven therapy. More research is needed to answer these intriguing questions and particularly to further refine early prediction and thus guide therapy at the individual level.

CONCLUSIONS / PRACTICAL IMPLICATIONS

This thesis demonstrates that treatment of early arthritis patients results in high remission rates, low bone damage and excellent functional status. However, full disease control including radiographic arrest in all patients remains an elusive target even in moderately active early arthritis. The fact that the majority of patients with radiographic progression were in DAS remission most of the time, points out that especially in early and less active arthritis patients other markers may be preferred to monitor disease activity.

A better disease control enhances the importance of identifying and treating co-morbid conditions associated with the underlying inflammatory disease. This thesis provides a

rationale for increased attention for cardiovascular risk in patients with RA and AS. Clinically, rheumatologists should be aware of the increased cardiovascular risk in their patients and should be encouraged to actively screen their population. Although still under debate, this thesis supports the idea of primary prevention of cardiovascular disease in patients with inflammatory diseases (by agents such as aspirin, statins or by early treatment of high blood pressure). In addition, cardiologists should recognize that rheumatic inflammatory disease is an important risk factor for cardiovascular disease. Eventually, patients with an inflammatory rheumatic disease should be made aware of the excess cardiovascular risk factor they run, and should be encouraged to improve their modifiable cardiovascular risk factors.

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DE STREKKING VAN DIT PROEFSCHRIFT

Er is steeds meer bewijs dat de mechanismen, die betrokken zijn bij zowel ontsteking, als aderverkalking, oftewel atherosclerose, vele overeenkomsten vertonen. In dit proefschrift worden effecten van ontstekingsremmende therapie op risicofactoren voor het krijgen van hart- en vaatziekten (meestal als gevolg van atherosclerose) onderzocht. Dit gebeurt bij patiënten met chronische gewrichtsontsteking, ook genoemd reumatoïde artritis (RA) en spondylitis ankylopoietica (SA). Enerzijds worden traditionele cardiovasculaire risicofactoren, zoals een afwijkend lipidenprofiel, insulineresistentie en afwijkende schildklierfunctie, onderzocht. Anderzijds wordt gekeken naar andere factoren, die het cardiovasculair risico zouden kunnen beïnvloeden, zoals microvasculaire disfunctie, surrogaatmarkers, zoals de intima-media dikte van de halsslagader en mogelijke nieuwe merkers voor het monitoren van de ziekteactiviteit bij SA. Het verhoogde cardiovasculaire risico bij patiënten met ontstekingsziekten heeft duidelijke relatie met (cumulatieve) ziekteactiviteit en lijkt te dalen wanneer de ontstekingsziekte zelf effectief wordt behandeld. Daarom is het belangrijk artritis zo vroeg en zo effectief mogelijk te behandelen en is het tweede hoofdonderwerp van dit proefschrift vroege artritis en de behandeling daarvan. Er wordt beschreven wat het nut is van een strak regime bij de behandeling van vroege gewrichtsontstekingen bij patiënten met relatief milde ziekteactiviteit. Tenslotte wordt de rol onderzocht, die de zogenaamde micropartikels spelen bij ontstekingprocessen in vroege RA.

CARDIOVASCULAIR RISICO BIJ PATIËNTEN MET SPONDYLITIS ANKYLOPOIETICA

Ook al is de beschikbare literatuur over het cardiovasculaire risico bij patiënten met SA beperkt, het idee heerst dat het ontstekingsproces bij SA onafhankelijk, dan wel gecombineerd met andere cardiovasculaire risicofactoren bijdraagt aan het ontstaan van atherosclerose. Er komt steeds meer bewijs dat functionele en structurele afwijkingen aan de (kleine) bloedvaten, zoals te meten is als endotheeldisfunctie, intima-media dikte of arteriële stijfheid, onafhankelijk van de klassieke cardiovasculaire risicofactoren, het risico op hart- en vaatziekten voorspellen. In de **hoofdstukken 1.1 en 1.2** hebben wij een verminderd functioneren van verschillende cardiovasculaire risicofactoren/surrogaat merkers geobserveerd: zowel microvasculaire functie, de rekruteerbaarheid van haarvaatjes in de huid, als de intima-media dikte van de halsslagader bleken afwijkend in negatieve zin bij patiënten met actieve SA ten opzichte van gezonde vrijwilligers. Deze verschillen bleven aantoonbaar ook na statistische correctie voor reeds bekende cardiovasculaire risicofactoren. Dit ondersteunt het idee dat deze surrogaatmarkers kunnen dienen voor de identificatie van patiënten met een verhoogd risico op het krijgen van hart- en vaatziekten.

Een andere belangrijke bevinding was, dat wanneer men de ontsteking behandelt met Tumor Necrose Factor (TNF) blokkerende therapie, zowel de microvasculaire functie, als de rekruteerbaarheid van haarvaatjes belangrijk verbeterde. Deze bevinding ondersteunt de hypothese dat ontsteking een rol speelt bij het verstoren van de microvasculaire functie.

Ondanks het feit dat atherosclerose een multifactorieel proces is, is een verstoord lipidenprofiel een van de belangrijkste risicofactoren voor het krijgen van hart- en vaatziekten. Het is al langer bekend dat ontsteking het lipidenprofiel nadelig kan beïnvloeden en er is ook aangetoond dat patiënten met RA of SA inderdaad een slechter lipidenprofiel hebben dan mensen zonder ontstekingsziekte. De laatste tijd is het echter ook gebleken dat niet alleen de absolute concentraties van de verschillende cholesterol fracties van belang zijn, maar dat ook de samenstelling van de individuele cholesterol eiwitten een rol lijkt te spelen, omdat deze samenstelling de eigenschappen van die deeltjes bepaalt. Heden ten dage is de zogenaamde high density lipoprotein (HDL) eiwit profilering een methode om naar de samenstelling van het HDL cholesterol te kijken. In **hoofdstuk 1.3** laten wij met behulp van deze techniek zien bij patiënten met SA, dat gedurende de behandeling met TNF blokkerende therapie, naast een verbetering van het lipidenprofiel, de samenstelling van het HDL deeltje daadwerkelijk verandert. Wij demonstreren dat het zogeheten serum amyloid A (SAA), wat een acute-fase eiwit is, dat vooral getransporteerd wordt in HDL en geassocieerd is met cardiovasculair risico, na behandeling met TNF blokkers verdwijnt van het oppervlak van het HDL deeltje, waardoor het deeltje meer beschermende eigenschappen tegen het krijgen van hart- en vaatziekten krijgt. Deze bevinding onderstreept nog eens dat het begrijpen van het effect van een bepaalde samenstelling van HDL deeltjes van belang is in relatie tot chronische ontstekingsziekten zoals SA.

De ziekteactiviteit bij SA wordt over het algemeen gemeten aan de hand van een score, de zogeheten BASDAI (Bath Ankylosing Spondylitis Disease Activity Index). Ondanks het feit dat de BASDAI een gevalideerde meetmethode is en in vele studies gebruikt wordt, blijft het een subjectieve meting, die gebaseerd is op een door de patiënt ingevulde vragenlijst. De laatste jaren hebben de TNF blokkerende middelen een vlucht genomen in de behandeling van SA. Omdat TNF blokkerende therapie niet zonder risico's is en het daarnaast ook buitengewoon kostbare medicijnen zijn, is het van het grootste belang vooraf patiënten te kunnen identificeren, die zeer waarschijnlijk wel of juist niet gunstig op het middel gaan reageren. Net zoals bij RA is het ook bij SA de wens om een meer objectieve parameter voor de ziekteactiviteit te hebben. Echter, zoals bij RA de C-reactive protein (CRP) en bezinking

(BSE) goede maten voor ontstekingsactiviteit zijn, is de bruikbaarheid van deze parameters bij SA meer controversieel. Daarom hebben wij in **hoofdstuk 1.4** gekeken naar de waarde van BSE, CRP, hsCRP en SAA bij het monitoren van de ziekteactiviteit en naar de samenhang van deze parameters met de BASDAI over de tijd. De gegevens uit deze studie laat zien dat de BASDAI niet, maar met name CRP en SAA wel, in staat zijn om zowel het effect van de TNF blokkerende therapie te monitoren, als om de patiëntgroep die een hoge kans hebben om goed te reageren op de therapie uit te selecteren.

CARDIOVASCULAIR RISICO BIJ PATIËNTEN MET REUMATOÏDE ARTRITIS

Hart- en vaatziekten zijn de belangrijkste oorzaak van oversterfte bij patiënten met RA. Dit verhoogde risico op hart- en vaatziekten is enerzijds veroorzaakt door een verhoogde aanwezigheid van de klassieke cardiovasculaire risicofactoren en anderzijds speelt (chronische) ontstekingsactiviteit hierbij een belangrijke rol.

Het “metabool syndroom” is het binnen een patiënt gezamenlijk voorkomen van risicofactoren voor zowel type 2 diabetes als voor hart- en vaatziekten (toegenomen buikvet, hyperglykemie, verstoord lipidenprofiel en hypertensie). Dit syndroom lijkt meer voor te komen bij patiënten met RA dan bij de gezonde vrijwilligers. Het hebben van het metabool syndroom verhoogd de kans op het krijgen van hart- en vaatziekten. Ook bij patiënten met hypothyreoïdie, een bekende risicofactor voor hart- en vaatziekten, is een relatie tussen metabool syndroom en het hebben van een verminderde functie van de schildklier beschreven. Hypothyreoïdie is een relatief veel voorkomende bijkomende ziekte bij mensen, die al RA hebben en recent is aangetoond dat het hebben van beide ziekten tegelijk geassocieerd is met meer hart- en vaatziekten. Daarom hebben wij onderzocht of metabool syndroom in verhoogde mate vóórkomt bij patiënten met zowel RA als hypothyreoïdie ten opzichte van patiënten met “alleen” RA. In **hoofdstuk 2.1** wordt beschreven dat de prevalentie van metabool syndroom inderdaad substantieel verhoogd bleek bij patiënten met zowel hypothyreoïdie als RA ten opzichte van patiënten met alleen RA. Aangezien het hebben van metabool syndroom het risico op het krijgen van een cardiovasculaire aandoening vergroot, zou dit een mogelijke verklaring kunnen zijn voor de eerder beschreven observatie, dat patiënten met zowel RA als hypothyreoïdie een nog meer uitgesproken risico hebben op het krijgen van hart- en vaatziekten dan RA patiënten zonder schildklierprobleem.

Een ander belangrijk mechanisme, dat ten grondslag ligt aan de ontwikkeling van atherosclerose, is het verminderd functioneren van de vaatwand, ook wel endotheeldisfunctie genoemd. Microvasculaire endotheeldisfunctie is niet alleen belangrijk bij

het ontstaan van orgaanschade in het hart of de nier, maar ook in de ontwikkeling van cardiovasculaire risicofactoren, zoals hypertensie en insulineresistentie. Bij patiënten met lang bestaande RA is eerder reeds microvasculaire disfunctie aangetoond. Een belangrijke vraag is wanneer in het ziekteproces de afwijkingen van functie van de (micro)circulatie ontstaan. In **hoofdstuk 2.2** bestuderen we de microvasculaire functie bij patiënten met vroege RA met lage systemische ziekteactiviteit, die nog niet behandeld werden met antireumatica, met als doel te onderzoeken of ook in deze fase al microvasculaire afwijkingen aantoonbaar waren. Wij observeerden in deze patiëntgroep een behouden endotheel-afhankelijke vaatverwijding en capillaire rekruteerbaarheid tijdens reactieve hyperemie. Dit suggereert dat meer ontsteking nodig is om microvasculaire afwijkingen te veroorzaken. In **hoofdstuk 2.3** beschrijven wij twee casus van RA patiënten, die tevens diabetes hadden, bij wie na het starten van de TNF blokker adalimumab de parameters voor de glucosehuishouding verbeterden. Gunstige effecten van behandeling van RA met TNF blokkerende medicijnen op diabetes waren nog niet eerder beschreven. TNF α wordt de laatste tijd wel sterk gekoppeld aan overgewicht en insulineresistentie en het wordt gesuggereerd dat TNF blokkers, bovenop hun sterke ontstekingsremmende werking, ook een gunstig effect zouden kunnen hebben op insulineresistentie bij reumatische ziekten. Ondanks dat onze bevindingen observationeel van aard zijn, suggereert dit dat TNF blokkade bij patiënten met RA en diabetes leidt tot verbeterde glucosehuishouding waarschijnlijk door het verbeteren van de insulineresistentie.

VROEGE ARTRITIS

Vroege en agressieve behandeling van RA is de laatste jaren toenemend succesvol gebleken, met name wanneer combinatietherapie met verschillende antireumatica, met soms ook TNF blokkerende medicijnen, werd toegepast. Tot de gunstige effecten behoren hoge percentages van (medicijnvrije) remissie rond de 40%, behoud van een goede functionaliteit in het dagelijks leven en een zo goed als complete stop op het voortschrijden van radiologisch gemeten schade. Om de betere resultaten, die nu worden verkregen ten opzichte van eerdere resultaten bij patiënten met veel langer bestaande RA te kunnen verklaren, werd het zogeheten "window of opportunity" concept geïntroduceerd. Het idee was dat het vroeg ingrijpen in het ontstekingsproces kon leiden tot gunstige uitkomst op de lange termijn. In **Hoofdstuk 3.1** wordt onderzocht of de in actieve RA beproefde aanpak van snelle en agressieve behandeling ook effectief is bij patiënten, die zich presenteren met een minder uitgesproken gewrichtsontsteking. Dit zijn de patiënten die normaliter op basis van hun ziekteactiviteit niet aan de criteria zouden voldoen om deel te nemen aan behandelonderzoeken bij actieve RA. In deze gerandomiseerde trial waarbij een actieve

behandelstrategie wordt vergeleken met de huidige “standaard” therapie, bleek dat de meeste patiënten uitstekende resultaten haalden wat betreft ziekteactiviteit: hoge remissie percentages, behoud van functionaliteit in het dagelijks leven en weinig röntgenschade. Dit was ongeacht de behandelgroep waarin de patiënt was gerandomiseerd. Gebaseerd op deze data kunnen wij daarom een meer agressieve aanpak dan de huidige, voor de behandeling van patiënten met een beperkt aantal gezwollen gewrichten, niet zonder meer aanbevelen. Deze studie laat zien dat het bewezen voordeel dat te behalen is met agressieve behandeling bij polyarthritis, bij milde arthritis niet zo duidelijk naar voren komt. Er was in beide groepen toch een klein aantal patiënten dat (toename van) röntgenschade liet zien. Aangezien deze patiënten allemaal behandeld werden met hoge doses methotrexaat en het grootste deel van de studie in klinische remissie waren, kan gesuggereerd worden dat de wereldwijd gebruikte DAS-gestuurde behandeling om toename van gewrichtsschade te verminderen, in deze patiëntgroep wellicht minder geschikt is dan bijvoorbeeld een behandelstrategie gebaseerd op röntgenschade.

In de laatste jaren hebben micropartikels (MP), wat kleine blaasjes zijn, die door lichaamscellen kunnen worden afgesnoerd, zich aangediend als een nieuwe schakel in de bevordering van ontstekingsprocessen. MP blijken geassocieerd te zijn met de activatie van complement, ontsteking en stolling bij verschillende ziekten, waaronder ontstekingsziekten. Het idee is dat hun aanwezigheid ontstekingsprocessen kan versterken. Waarschijnlijk stimuleren de MP ontsteking op verschillende manieren, zoals via de activatie van cellen in de vaatwand en witte bloedcellen, de productie en het vrijkomen van verschillende stoffen betrokken bij ontsteking en door het activeren van het complementsysteem, wat een erg belangrijke rol in het ontstaansproces van RA speelt. Aan de andere kant zou ontsteking zelf de vorming van MP kunnen stimuleren. Zo zijn er laboratorium- en muizenstudies verricht waarbij toedienen van ontstekingseiwitten leidde tot een toename van MP aantallen. Hoewel het dus zo is dat ontsteking de vorming van MP stimuleert en dat MP zelf ontsteking kunnen veroorzaken, dan wel versterken, is het vooralsnog onbekend of circulerende MP vooral een exponent zijn van bestaande ontsteking of dat MP daadwerkelijk bijdragen aan de ontwikkeling van ontsteking of ontstekingsziekte. In **hoofdstuk 3.2** hebben wij onderzocht of het aantal MP in bloedplasma samenhangt met de ontstekingsactiviteit, door MP in zeer vroege nog onbehandelde RA patiënten te vergelijken met gezonde vrijwilligers. Verder hebben we naar de effecten gekeken van agressieve ontstekingsremmende combinatietherapie (de COBRA strategie) op het aantal MP en de samenstelling ervan. Deze studie toont aan dat het aantal MP, dat de complement componenten C1q, CRP of SAP op het oppervlak draagt, verhoogd is in vroege actieve RA. Ook al werd de ziekteactiviteit bij

deze patiënten sterk onderdrukt met de eerder genoemde therapie, de aantallen en samenstelling van de MP bleef ongewijzigd. Deze observatie suggereert dat ontsteking niet de onderliggende oorzaak is van de vorming van MP bij deze patiënten. Onze belangrijkste bevinding, dat het krachtig remmen van ontsteking in staat was de ziekteactiviteit te verlagen, maar dat de samenhang tussen circulerende MP en de activatie van het complementsysteem blijft bestaan, suggereert dat zowel MP, als complement, actief bijdragen aan de ontwikkeling en/of het chronische karakter van ontstekingsziekten, zoals RA.

TOEKOMSTIG ONDERZOEK

Ontsteking wordt gezien als de ontbrekende schakel om het toegenomen risico op hart- en vaatziekten bij mensen met reumatische ontstekingsziekten te verklaren. Ontsteking zou, direct, dan wel indirect, dit verhoogde cardiovasculair risico kunnen verklaren. Toekomstig onderzoek zou zich moeten toespitsen op het verder ontrafelen van de wijze waarop ontsteking in het algemeen en bij reumatische ontstekingsziekten in het bijzonder leidt tot dit verhoogde cardiovasculaire risico. Sommige studies rapporteren samenhang tussen cardiovasculaire surrogaatmarkers en aanwezige ontstekingsparameters die actuele ontstekingsactiviteit weerspiegelen, maar niet met ontstekingsparameters, die meer een chronische ontstekingsproces weergeven. Andere studies tonen het omgekeerde. Het zou daarom interessant zijn om te onderzoeken of de bijdrage van “acute” versus “chronische” ontsteking van elkaar verschilt. Daarbij is een interessante vraag of en zo ja op welk moment eventuele nadelige effecten op het cardiovasculair systeem nog omkeerbaar zijn. Observationele studies demonstreren dat patiënten met gewrichtsontsteking, die goed reageren op ontstekingsremmende therapie (zoals TNF blokkerende therapie en methotrexaat), een lager risico op hart- en vaatziekten en een verbeterd cardiovasculair risicoprofiel hebben. Deze bevindingen ondersteunen de behoefte aan verder onderzoek op dit vlak om de mechanismen waarmee ontsteking invloed uitoefent op het cardiovasculair risico te ontrafelen en op welke wijze effectieve ontstekingsremmende therapie gunstig, dan wel ongunstig zou kunnen zijn voor het ontstaan van aderverkalking. Samengevat is het van essentieel belang om in te zien dat de mechanismen die een rol spelen bij atherosclerose en reumatische ontstekingsziekten grotendeels overeenkomen, om zo verder te kunnen komen in het begrip ten aanzien van de verhoogde cardiovasculaire ziektelast in deze populatie.

De in dit proefschrift gepresenteerde bevinding dat een deel van de patiënten met vroege gewrichtsontsteking, ondanks een zeer lage ziekteactiviteit gedurende het grootste deel van

de studie, gemeten aan de DAS-score, toch toename van gewrichtsschade lieten zien, suggereert dat in deze patiëntgroep wellicht andere maten dan de DAS-score beter zijn om de behandeling op af te stemmen. Zo zou bijvoorbeeld een therapie die stuurt op structurele schade de voorkeur kunnen hebben boven therapie gestuurd op ziekte-activiteit in deze patiëntgroep. Uiteraard is nieuw onderzoek nodig om deze vraag te beantwoorden. Daarnaast is het van belang meer specifiek onderzoek te doen naar manieren om zo vroeg mogelijk in het ziekteproces te voorspellen wat de waarschijnlijke uitkomst van een patiënt zal gaan zijn en zo uiteindelijk te komen tot een op maat gesneden behandelstrategie op individueel niveau.

CONCLUSIES/ PRAKTISCHE TOEPASSINGEN

Uit dit proefschrift blijkt dat de ziekteactiviteit bij patiënten met vroege milde artiritis over het algemeen goed te onderdrukken is, maar dat volledige controle over de ziekte, waaronder het totaal stoppen van röntgenshade bij alle patiënten vooralsnog een utopie blijft. Een betere controle over de ontstekingsziekte zelf nodigt uit tot het opsporen en behandelen van comorbiditeit, die in verband staat met deze ontstekingsziekte. Dit proefschrift draagt bij aan de kennis dat het hebben van een ontstekingsziekte, zoals RA of SA, het risico op het krijgen van hart- en vaatziekten vergroot. Daarmee levert het een rationale voor speciale aandacht voor het risico op hart- en vaatziekten bij patiënten met RA en SA. Reumatologen zouden op de hoogte moeten zijn van dit verhoogde cardiovasculaire risico en aangemoedigd moeten zijn om in deze patiëntpopulatie actief te screenen op risicofactoren. Dit is met name van belang, omdat met het beter onder controle kunnen houden van de ontstekingsziekten zelf, wat op zich de ontwikkeling van aderverkalking op zijn minst zou kunnen vertragen, ook de kwaliteit van leven voor patiënten spectaculair is verbeterd en het specifiek behandelen van comorbiditeit extra belangrijk wordt. In lijn hiermee, ondersteunt dit proefschrift ook de gedachte, dat primaire preventie van hart- en vaatziekten bij deze patiëntgroepen met middelen als bijvoorbeeld aspirine, statines of het laagdrempelig behandelen van een te hoge bloeddruk, zinvol kan zijn. Voor cardiologen is het van belang in te zien dat het hebben van een reumatische ontstekingsziekte een belangrijke risicofactor is voor het krijgen van hart- en vaatziekten. Tenslotte, zouden de patiënten zelf op de hoogte moeten zijn van hun verhoogd cardiovasculair risico, opdat zij extra gemotiveerd zijn om hun beïnvloedbare risicofactoren aan te pakken en daarmee te verbeteren.

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CURRICULUM VITAE

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